Pollination Ecosystem Services to Onion Hybrid Seed Crops in South Africa

by

Mariëtte Rieks Brand

Dissertation presented for the degree of Doctor of Philosophy (Science)
in the Faculty of AgriSciences at Stellenbosch University

Promoter: Prof.Michael J. Samways

Co-promoters: Dr. Ruan Veldtman

Dr. Jonathan F. Colville

April 2014

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

April 2014

Abstract

Insect pollination contributes in various degrees toward the production of a variety of agricultural crops that ensure diversity and nutritional value in the human diet. Although managed honeybees (*Apis mellifera* L.) are still the most economically valuable pollinators of monoculture crops cultivated globally, wild pollinator communities can contribute substantially toward crop pollination through pollination ecosystem services sourced from neighbouring natural habitats. Pollination ecosystem services are thus valuable and can motivate for the protection of natural ecosystems hosting diverse insect pollinator communities. F_1 onion hybrid seed production is entirely dependent on high insect pollinator activity to ensure cross pollination, seed set and profitable seed yields. Data was collected on 18 onion hybrid seed crops grown in the semi-arid Klein Karoo and southern Karoo regions of the Western Cape, South Africa. These two main production regions are located within the Succulent Karoo biome, recognized as a global biodiversity hotspot of especially high plant diversity. It is also habitat to the indigenous Cape honeybee (*Apis mellifera capensis* Esch.). Sites selected varied in the percentages of available natural habitat and managed honeybee hives stocking density. Diverse anthophile assemblages were sampled with pan traps within all the onion fields, regardless of the percentage of available natural habitat near the crop. Crop management practices significantly affected the diversity of anthophile species caught within onion fields, although less than 20% of this diversity was observed actually visiting onion flowers. The honeybee (managed and wild) was by far the most important pollinator because of its high visitation frequency and regular substantial onion pollen loads carried on their bodies. Honeybee visitation significantly increased onion hybrid seed yield, while anthophile diversity and non-*Apis* visitation had no effect on seed yield. Neither managed hive density, nor percentage natural habitat were important in determining honeybee visitation or seed yield. Total annual rainfall was the only significant factor determining honeybee visitation. Secondary factors caused by rainfall variability, such as wild flower abundance or soil moisture, may have significantly affected honeybee visitation. In addition, the positive correlation between honeybee visitation and the diversity of hand-sampled insects from onion flowers; indicate that either or both onion varietal attractiveness and/or pollinator population size may have had significant effects on overall insect visitation. Honeybees showed marked discrimination between hybrid onion parental lines and preferred to forage on one or the other during single foraging trips. Hybrid onion parents differed significantly in nectar characteristics and onion flower scent which would encourage selective foraging through floral constancy. Interspecies interactions were insignificant in causing

increased honeybee pollination because of the scarcity of non-*Apis* visitors. Most farming practices are subjected to favourable environmental conditions for successful production. However, and especially in the South African context, the dependence of onion hybrid seed crops on insect pollination for successful yields, increase its reliance on natural ecosystem dynamics that may deliver abundant wild honeybee pollinators, or attract them away from the crops. Nevertheless, this dependence can be mitigated effectively by the use of managed honeybee colonies to supplement wild honeybee workers on the flowers.

Opsomming

Insek bestuiwing dra in verskillende grade by tot die produksie van landbou gewasse wat variteit en voedingswaarde in die mens se dieet verseker. Al is die heuningby (*Apis mellifera* L.) steeds die waardevolste ekonomiese bestuiwer van verboude enkelgewasse, kan wilde bestuiwers wesenlik bydra tot gewasbestuiwing deur middel van ekosisteem dienste afkomstig van natuurlike habitatte. Bestuiwing ekosisteem dienste is daarom waardevol en kan dus die bewaring van natuurlike ekosisteme, wat diverse gemeenskappe huisves, regverdig. F_1 basterui saadproduksie is totaal afhanklik van hoë insek-bestuiwer aktiwiteit om kruisbestuiwing, saadvorming en winsgewende saadopbrengste te verseker. Data is ingesamel op 18 basterui saad aanplantings in die half-droë Klein Karoo en suid-Karoo streke van die Weskaap, Suid-Afrika. Hierdie twee hoof produksie streke is geleë binne die Sukkulente Karoo bioom wat erken word as 'n globale biodiversiteits "hotspot" met hoë plant diversiteit. Dit is ook die habitat van die inheemse Kaapse heuningby (*Apis mellifera capensis* Esch.). Aanplantings is gekies om verskillende grade van beskikbare natuurlike habitat en bestuurde heuningby korf digthede te verteenwoordig. Diverse versamelings blom-besoekers is versamel met water-wippe in al die aanplantings, ongeag die persentasie natuurlike habitat beskikbaar by elke aanplanting. Gewas bestuurspraktyke het die diversiteit van blom-besoekers betekenisvol beïnvloed. Tog is minder as 20% van hierdie diversiteit as aktiewe besoekers op die uiekoppe waargeneem. Heuningbye (bestuur of wild) was oorwegend die belangrikste bestuiwers as gevolg van hoë besoek frekwensies en wesenlike ladings uiestuifmeel op hulle liggame. Heuningby besoeke het saadopbrengs betekenisvol verhoog, maar blom-besoeker diversiteit en nie-*Apis* besoeke het geen effek op saadopbrengs gehad nie. Bestuurde korf digtheid en persentasie natuurlike habitat was nie belangrik in die bepaling van heuningby besoeke of basterui saadopbrengste nie. Totale jaarlikse reënval was die enigste betekenisvolle faktor wat heuningby besoeke bepaal het. Sekondêre faktore wat versoorsaak word deur reënval veranderlikheid, soos veldblom volopheid of grondvog, kon betekenisvolle effekte op die aantal heuningby besoeke gehad het. Bykomend, dui die positiewe korrelasie tussen heuningby besoeke en die diversiteit van hand-versamelde insekte vanaf die uiekoppe op die moontlike betekenisvolle effek van elk of beide basterui variteit aantreklikheid en/of bestuiwer populasie grote op algehele insek besoeke. Heuningbye het noemenswaardige diskriminasie getoon tussen die basterui ouerlyne en het verkies om op een of die ander te wei tydens enkele weidingstogte. Basterui ouerlyne het betekenisvol verskil in nektar eienskappe en blomgeur wat die selektiewe weiding van heuningbye, toegepas deur blomkonstantheid, sal aanmoedig. Tussen-spesie interaksies was

onbetekenisvol in die verhoging van heuningby bestuiwing omdat nie-*Apis* besoekers baie skaars was. Meeste boerdery praktyke is onderhewig aan gunstige omgewings toestande vir suksesvolle produksie. Maar, en veral in die Suid-Afrikaanse konteks, omdat basterui saad aanplantings afhanklik is van insek bestuiwing vir suksesvolle opbrengste, word daar meer staat gemaak op natuurlike ekosisteem dinamika wat volop wilde heuningby bestuiwers kan voorsien, of selfs bestuiwers van die aanplanting kan weg lok. Nietemin, hierdie afhanklikheid kan effektief verlaag word deur die gebruik van bestuurde heuningby kolonies om die aantal wilde heuningby werkers op die blomme aan te vul.

Acknowledgments

I want to express my sincere gratitude to:

- the Global Pollination Project and the South African National Biodiversity Institute for funding and supporting this project,
- Peter Greeff from the Nelson Mandela Metropolitan University for valuable advice at the initial stages of the project,
- Klein Karoo Seed Production and other seed companies for their involvement, support and assistance in selecting appropriate crops for data collection and making contact with the farmers,
- all the farmers involved for permission to work on their land and supplying valuable information,
- Fanie the beekeeper for assisting with equipment and information,
- Terence Suinyuy from the University of KwaZulu-Natal for the sampling and analysis of onion flower scent,
- Sue Nicolson from Pretoria University for advising on nectar sampling techniques, making equipment available for data sampling, and editing Chapter 5,
- the following people who helped with species identification: Connal Eardley (Apoidea), Fredrich Gess (Wasps), Neal Evenhuis (Bombyliidae), Jonathan F. Colville (Scarabaeidae), Axel Ssymank (Syrphidae), Pierfilippo Cerretti (Tachinidae), Daniel Whitmore (Sarcophagidae), Knut Rognes (Calliphoridae), Marcia Couri (Muscidae), Jason Londt (Asilidae), Marco A. Bologna (Meloidae)
- the field workers for your assistance and perseverance in the hot Karoo sun: Kate Smorenberg, John-Hugh Marshall, Coenie Brand, Lizette Crous, Melanie Coetzer, Buthle Mambikimba

Contents

Abstract i

Opsomming iii

Acknowledgments v

1 GENERAL INTRODUCTION

Ecosystem services: a conceptual framework for environmental protection 1

Pollination ecosystem services & biodiversity: state and trends of wild and managed pollinators 4

Insect pollination is critical in onion hybrid seed production 7

The South African seed industry 13

The study area and its pollinator community 14

Research objectives 19

References 21

2 ANTHOPHILE DIVERSITY AND ABUNDANCE WITHIN ONION HYBRID SEED CROPS IN SOUTH AFRICA: GRADIENTS OF PERCENTAGE NATURAL HABITAT AND DIFFERING FARM MANAGEMENT PRACTICES

Abstract 32

Introduction 32

Methods 34

Results 41

Discussion 46

Conclusion 49

References 51

3 WHICH INSECT GROUPS ARE EFFECTIVE POLLINATORS OF ONION HYBRID SEED CROPS IN SOUTH AFRICA?

Abstract 72

Introduction 73

Methods 75

Results 79

Discussion 81

Conclusion 86

References 87

4 ONION HYBRID SEED PRODUCTION IN SOUTH AFRICA: DOES IT BENEFIT FROM POLLINATION ECOSYSTEM SERVICES?

Abstract 103

Introduction 103

Methods 106

Results 109

Discussion 110

Conclusion 113
References 114

5 HONEYBEE FORAGING PATTERNS ON ONION HYBRID SEED CROPS IN SOUTH AFRICA: FLORISTIC AND BIOTIC FACTORS THAT MIGHT INFLUENCE CROSS-POLLINATION

Abstract 124
Introduction 125
Methods 129
Results 133
Discussion 135
Conclusion 139
References 141

6: GENERAL DISCUSSION 156

References 164

Appendix A: Farm sites used for data collection vi

Appendix B: Species checklist xi

1

GENERAL INTRODUCTION

ECOSYSTEM SERVICES: A CONCEPTUAL FRAMEWORK FOR ENVIRONMENTAL PROTECTION

Since the economic revolution in the 1950's, Earth's ecosystems have become increasingly dominated by humanity and few have escaped some degree of transformation through human actions (Millennium Ecosystem Assessment (MEA) 2005). Land-use changes, introduced species, exploitation of terrestrial and marine resources, alteration of biochemical cycles and increased pollution have been significant anthropogenic drivers of biodiversity change and loss (Vitousek *et al.,* 1997; Leadley *et al.,* 2010). Species extinctions are a natural process and have always been associated with the procession of life on Earth. However, current extinction rates are much higher than rates suggested by the fossil record (Pimm *et al.,* 1995). Habitat loss, soil loss, increasing domestic and toxic waste, pesticides, water and air pollution, acid rain, global warming and a growing diet of regular meat consumption are real threats to a healthy, life-supporting Earth that is essential in fulfilling human livelihoods (Cremo and Goswani, 1995; Tilman *et al.*, 2001).

Apart from obvious environmental degradation and increases in the number of environmental disasters, concepts describing the human-nature relationship have been formulated to further augment social environmental consciousness. One such concept, the Ecological Footprint (EF), developed by William Rees and Mathis Wackernagel during the early 1990's aims to measure human demand on the Earth's ecosystems (Rees, 1992; Wackernagel, 1994). The latest figures measured the human ecological footprint at 2.7 gha (global hectares) per person in 2007, while the Earth's bio-capacity (regeneration capacity) was measured at only 1.8 gha per person, which represents an overshoot of 50 per cent (WWF, 2010). In other words, people used an amount of resources equivalent to 1.5 planet Earths in 2007 to support their activities, and this figure is projected to be 2 planets by the year 2030.

A second measure of sustainable use of the environment is the Environmental Sustainability Index (ESI), based on a compilation of 21 indicators of environmental stewardship practices fostered by the world's nations (Esty *et al.,* 2005). The main aim of the ESI is to provide comparative analysis that renders environmental management more

quantitative, empirically grounded and systematic in order to facilitate environmental policy and decision-making at a national level (Esty *et al.,* 2005).

A third, widely used and practical concept which has become increasingly popular is that of ecosystem services (ES), which highlights the utilitarian rather than intrinsic value of ecosystems (MEA, 2005b). The ES concept is a model that links the functioning of ecosystems to human welfare (MEA, 2005b; Fisher *et al.,* 2009) and in doing so, builds a case for conserving "natural capital". The ES concept regards the world's ecosystems as capital assets that will, if properly managed, continue to supply a flow of vital services to human populations (Daily, 2000). Until fairly recently, economic activity around ecosystem capital was comparatively limited, with an abundance of ecosystem capital supplying 'free' ecosystem services (Daily, 2000). However, the increase in threats to, and depletion of, these 'free' services compelled ecologists and economists to join forces to take stock of our natural assets and express its value in monetary terms (Daily, 2000).

There have been numerous attempts by ecological economists to find a meaningful and consistent definition of ES, and to set the different services provided to us by nature into logical classification schemes (Costanza *et al.*, 1997; Daily, 1997; de Groot *et al.,* 2002; MEA, 2005b; Boyd & Banzhaf, 2007; Wallace, 2007; Fisher & Turner, 2008; Fisher *et al.,* 2009). A clear definition of ES would facilitate meaningful decisions in natural resource management (Wallace, 2007) and enable comparisons across different projects, policy contexts, and time and space (Fisher *et al.,* 2009). Fisher *et al.* (2009) provide a summary and analysis of the different definitions that have been given in the past by various authors to describe ES, and they themselves suggest the following definition: "ecosystem services are the aspects of ecosystems utilized (actively or passively) to produce human well-being". The Millennium Ecosystem Assessment (MEA, 2005b) identified twenty four such services and divided them into four main categories namely provisional and cultural (direct), and supporting and regulating (indirect) ES.

The main purpose driving the valuation of ES in monetary terms is to capture it in commercial markets and increase the chances for it to be considered in policy decisions (Costanza *et al.,* 1998). However, counter arguments to this strategy claim that the commercialization of the relationship between humans and nature – as a consequence of the biological sciences conforming to neoliberalism – are not sustainable (Buscher, 2008). Modern societies are built on assumptions of infinite resources (Lamm, 2006). Contrary to

the current strategy of reinvention and radical reconfiguration observed in conservation biology (Meine *et al.,* 2006; Buscher, 2008), the ultimate solution would be for economic theories to adapt to ecological realities (Lamm, 2006). However, as Daily (2000) puts it "valuation is merely a tool in the much larger politic of decision-making – it is a way of organizing information to help guide decision making, but not a solution in itself".

Ecosystem services projects currently succeed in securing more funding towards conservation projects, as opposed to biodiversity focused projects (Goldman *et al.,* 2008). However, even though the amount of research on ES has grown substantially in recent years, few studies embrace stakeholder empowerment to enable on-the-ground management of areas delivering ES (Cowling *et al.,* 2008). ES research should not be characterized by technological sophistication and societal irrelevance, but should be geared for implementation and respond to stakeholder needs (Cowling *et al.,* 2008). Indeed, ES research is the cornerstone of sustainability science (Clark & Dickson, 2003).

Several stages or phases contribute to the evolution of the theory and conceptual framework of ES to practical integration in decision-making and ultimately accomplish the effective and adaptive management of ES in the quest for sustainability (Daily & Matson, 2008). Daily (1997, 2000) emphasised four key elements in achieving this goal

1. Identifying ES – taking stock of natural capital by systematically and quantitatively cataloguing the sources and consumers of ES
2. Characterizing ES
    a. *ecologically*: describing how ecosystems generate services and the level of services supplied in terms of quality and quantity
    b. *economically*: determining the importance or value of services, using ecological attributes
3. Safeguarding ES – by establishing the institutional means to secure decision-making and implementation
4. Monitoring ES – to evaluate the efficiency of the safeguards

Cowling *et al.* (2008) proposed a pragmatic operational model for achieving the safeguarding of ES by internalizing resource management goals into economic sectors and development models, policies and programs; in other words, mainstreaming ES. The model has three phases; assessment, planning and management. In the assessment phase, the model implements three separate assessments: social, biophysical and economic (valuation), each of

which should engage the participation of experts in the discerned disciplines. Heal (2000) points out that the biophysical assessment provides the knowledge-based case for safeguarding services, rather than the valuation assessment, which depends on information generated by the other two assessments (Cowling *et al.,* 2008).

Furthermore, although much is known of general ecosystem functioning and the supply of ES in general, information on local ecosystems and their supply of services is lacking (Daily, 2000). This lack of local information limits the incorporation of natural capital into local decision-making (Cowling *et al.,* 2008; Daily & Matson, 2008).

POLLINATION ECOSYSTEM SERVICES & BIODIVERSITY: STATE AND TRENDS OF WILD AND MANAGED POLLINATORS

Pollination is a regulating ecosystem service that regulates plant reproduction through the movement of pollen and many wild angiosperm populations, including several agricultural crops which depend on it for existence and persistence (MEA, 2005c). Animals are important pollinators for most angiosperms, while one third of the food humans eat is either directly or indirectly dependent on animal pollination for production (O'Toole, 1993; Richards, 1993). Eighty seven global food crops are dependent on animal pollination (Klein *et al.,* 2007) for both quality and yield security.

Bees are generally considered to be the most important group of animal pollinators (Williams *et al.,* 2001; Shepherd *et al.,* 2003) since they are totally dependent on floral resources for food provision in all life stages (Buckmann & Nabhan, 1996). Several other insect taxa are also adapted to feed on floral resources to varying degrees. These include pollen wasps (Vespidae: Masarinae) (Gess, 1996), certain fly families (Bombyliidae, Nemestrinidae, Tabanidae and Syrphidae), several beetle families (Bernhardt, 2000), butterflies and moths (Lepidoptera) (Ehrlich & Raven, 1964).

However, there have been recent concerns about a global decline of pollinator abundance (Buckmann & Nabhan, 1996; Allen-Wardell *et al.,* 1998), which have been confirmed by several reports around the globe (Watanabe, 1994; Biesmeijer et al., 2006; Kluser & Peduzzi, 2007; Kosior et al., 2007; Oldroyd, 2007). Nevertheless, Aizen *et al.* (2008) found no evidence of a shortage of pollination to pollinator-dependent agricultural food crops in both the developed as well as the developing worlds, because the production of these crops has

increased steadily between 1961 and 2006. Even though the global number of managed honeybee colonies has increased by about 45% during the past 50 years, data also show that the proportion of pollinator-dependent crops cultivated globally increased at a rate of 300% during the same time, a trend that is more pronounced in the developing than in the developed world (Aizen *et al.,* 2009). This scenario suggests that the demand for pollination is most likely to increase if the trend continues, putting pressure on pollinator populations. Assuming a scenario of a total loss of pollinators, Aizen *et al.* (2009) found that total agricultural production worldwide would decrease by about 3-8%, with a lesser effect on the diversity of crops produced. There is also a prediction that the demand for agricultural land will increase as pollinators decline, especially in developing worlds, in order to compensate for production deficits (Aizen *et al.,* 2009).

The simultaneous or mass flowering of hundreds of hectares of cropland and orchards poses a challenge to any natural assemblage of pollinators, especially in cases of intensive agriculture. Therefore, most crops are greatly dependent on a single domesticated and managed species for pollination services – the European honeybee ('Honeybee', or 'honeybees' for several individuals) (*Apis mellifera* Linnaeus) (Free, 1993a). The Honeybee can easily be supplied in great numbers to a wide variety of crop plants for pollination. However, there are dangers in relying on a single species for pollination services. Honeybee colonies for example are susceptible to a number of diseases (Johannsmeier, 2001), and colony numbers can be rapidly reduced when hives become infected. Large-scale periodic die-offs of managed honeybee colonies have been reported in the United States and Europe since the beginning of the 21th century, a phenomenon that has been named Colony Collapse Disorder (CCD) and driven by unknown causes (Oldroyd, 2007). Here in South Africa, a particularly virulent disease, American Foul Brood (AFB) has recently been diagnosed in Western Cape apiaries, and has the potential of causing wide-scale and significant losses to the region's beekeeping activities (Allsopp, 2009; Steyn, 2009), and threaten the survival of wild honeybee colonies indigenous to the region. Thus, as with natural ecosystems, agriculture would benefit from a diversity of pollinator species to deliver pollination ecosystem services (PES hereafter).

Large scale commercial crop production threatens biodiversity and has negative impacts on pollinator diversity and abundance (Donaldson *et al.,* 2002; de Ruijter, 2002). Klein *et al.* (2007) reviewed sixteen studies on the effect of agricultural intensification on crop

pollination at local and landscape scale. All the studies showed that agricultural intensification and the degradation of habitat quality had a negative impact on pollination. The impact on pollinator populations remains largely unknown where chemical fertilizers, herbicides and pesticides are applied intensively. However, a recent study by Gill *et al.* (2012) showed that a combined effect, after chronic exposure to two commonly used pesticides, caused impaired foraging behaviour and increased worker mortality in bumble bees. Increasing mechanization and the cultivation of large areas with mono-culture crops fragments and destroys suitable pollinator habitat. It has been shown that increased habitat fragmentation can alter pollinator assemblages (Aizen & Feinsinger, 1994; Donaldson *et al.,* 2002; Harris & Johnson, 2004; Brosi *et al.,* 2008). Cultivated crops have become increasingly dependent on external inputs which include the services of managed pollinators.

However, a current trend is to adapt agricultural practices to be more sustainable and eco-friendly, which is captured by the term agro-ecosystems (Altieri, 1995, 1999). As far as pollination is concerned, a number of different ways to increase the use of this ecosystem service in agro-ecosystems have been explored. Several authors assessed the value of conserving natural habitat surrounding plantations or crop fields to supply an increased diversity of potential pollinators (Heard & Exley, 1994; Kremen *et al.,* 2004, 2002; Klein *et al.,* 2003a, 2003b; Ricketts, 2004; Ricketts *et al.,* 2004; De Marco & Coelho, 2004; Blanche & Cunningham, 2005; Morandin & Winston, 2005; Blanche *et al.,* 2006; Chacoff & Aizen, 2006; Greenleaf & Kremen, 2006a, 2006b). In northern California, USA, Kremen *et al.* (2002, 2004) showed that organic watermelon crops with more than 40% natural habitat within a radius of 2.4 km (maximum bee foraging range) received full pollination services from native bee species and could rely entirely on native bees for pollination. Furthermore, much smaller proportions of natural habitat near crops were still able to provide some contribution to watermelon pollination from native bees (Kremen *et al.,* 2002, 2004). In sunflower, Greenleaf & Kremen (2006a) found that both proximity to natural habitat and crop planting practices significantly influenced the pollination services provided directly and indirectly by wild bees, also in northern California. Generally, all the studies highlighted the fact that pollinator diversity plays an important role in sustaining pollination services, as fluctuations in populations of different pollinator species occur annually (Kremen *et al.,* 2002, 2004).

Gallai *et al.* (2009) reported the total economic value of pollination worldwide which amounted to 158 billion Euros (USD 209 billion, 1 May 2012). Pollinator-dependent crops

are on average five times more valuable per ton than crops not dependent on animal pollination (Gallai *et al.,* 2009). Therefore, pollinator conservation has globally been identified as a priority due to this guild fulfilling an important ecosystem function, not only by maintaining indigenous floral biodiversity, but also in providing PES to cultivated crops, especially fruits and vegetables, which ultimately contribute to sustainable agriculture and food security.

To endorse the conservation and sustainable management of pollinators and their habitats for its value as ecosystem service providers to agriculture, the International Initiative for the Conservation and Sustainable Use of Pollinators (also known as the International Pollinators Initiative (IPI)) was established in 2000 at the Fifth Conference of Parties (COP V) piloted by the Plant Production and Protection division (AGP) of the Food and Agriculture Organization of the United Nations (FAO) (www.fao.org). The Plan of Action of the IPI was established at the Sixth Conference of Parties and consists of four elements: 1) assessment, 2) adaptive management, 3) capacity building and 4) mainstreaming. The FAO - AGP's Global Action on Pollination Services for Sustainable Agriculture program assists participating countries to formulate policies and utilize and conserve PES to ensure sustainable agro-ecosystems by providing tools and guidance. A collaboration between the FAO, the Global Environment Facility (GEF) and the United Nations Environment Programme (UNEP) brought about the initiation and execution of the Global Pollination Project that aims to identify practices and build capacity in the management of PES (www.internationalpollinatorsinitiative.org). Seven countries are participating in the project that runs from 2009 to 2013 and these include Brazil, Ghana, Kenya, India, Nepal, Pakistan and South Africa. The work presented in this thesis reports the results of research that focused on PES delivered to onion hybrid seed crops in South Africa, one of several projects funded by the Global Pollination Project participants.

INSECT POLLINATION IS CRITICAL IN ONION HYBRID SEED PRODUCTION

The onion (*Allium cepa* L.), a member of the Alliaceae family, is a biennial plant, requiring two growing seasons to complete the cycle from seed to seed. Bulbs are ready for harvesting after the first growing season, after which the plant will initiate the flowering process. Bulb onions are commercially grown as annual crops because the whole plant is harvested after the

first growing season. The biennial cycle of onion seed production consists of two phases, the seed-bulb phase in the first year and the bulb-seed phase in the second year.

It is unclear from where onions originated. Jones & Rosa (1928) mention that the onion no longer grows in the wild, and that the onion is most probably native to the region stretching from Palestine to India. Vavilov (1951) suggested that onions probably originated in Central Asia. Onion seed is mainly produced in regions with reliable warm, dry summers with low atmospheric humidity to reduce the risk of diseases (Brewster, 2008). Warm, clear weather also promotes optimal insect pollination activity on blooming crops (Brewster, 2008).

Hybrid onion cultivars have been developed using cytoplasmic male sterility (George, 1999) and was first explored by Jones & Clarke (1943). F_1 hybrid onion cultivars have been fixed in recent years through the establishment of cross breeding parental lines and are now a dominant trend in onion breeding (Brewster, 2008). Three parental lines are used and maintained for F_1 hybrid cultivars namely A, B and R. Line A is the male-sterile parent with deformed or suppressed anthers that result in the absence of pollen (Wills & North, 1978). Line B is the maintainer line used to maintain male-sterility in line A, and the restorer line (R) is the male-fertile parent with anthers that produce viable pollen (Shanmugasundaram, 1998; Pathak, 2000). Hybrid seed is produced when line A and R are crossed (Shanmugasundaram, 1998; Pathak, 2000). Hybrid cultivars are superior to open pollinated cultivars in that they produce higher yields, larger bulb sizes and more uniform bulbs (Shanmugasundaram, 1998). Bulb onion cultivars are grouped into short, intermediate and long-day types (Shanmugasundaram, 1998), while seed growers in South Africa mainly cultivate short day onion cultivars.

General guidelines for onion hybrid seed production recommend the planting of 600 000 bulbs per hectare with a male-fertile to male-sterile ratio ranging between 1:3 to 1:8 (George, 1999). Insect pollinators are essential for the pollination of F_1 hybrid cultivars and serve as pollen vectors that carry pollen from male-fertile to male-sterile flowers to bring about pollination and ensure the production of viable seeds (Brewster, 2008). In fact, the bee factor for onions is 95% (Johannsmeier, 2005). Controlled pollination is also fundamental in onion breeding programs (Brewster, 2008).

Onion is a highly cross-pollinated crop and cross-pollination between cultivars is common (Free, 1993; Shanmugasundaram, 1998). Therefore, isolation distances between different onion cultivars is needed to prevent genetic contamination by foreign pollen.

Recommendations on isolation distances ranges between 0.8 km and 5 km (Free, 1993). But Jones & Mann (1963) suggested that complete isolation would be virtually impossible because of the large foraging distances covered by some pollinator insects. They suggest that different localities should be dedicated for certain cultivars only and should be separated by at least 4.8 km.

The onion plant (*Allium cepa* L.) produces flower heads on single elongated inflorescence stalks (scapes) which are 1-2 meters long. The flower colour is white, green or striped, depending on the cultivar. Onion inflorescences or umbels carry between 200 and 600 small flowers (3-4 mm in length), depending on the cultivar and growing conditions. The flowers are made up of parts of three of each floral organ in five separate whorls, the outer perianth, inner perianth, outer stamens, inner stamens and the carpels (ovaries) (see Figure 1.1). The superior carpels are fused with two ovules per carpel (Free, 1993b). The style elongates from 1 mm to 5 mm only after the stamens dehisced (Free, 1993b).

Only a few flowers open on the umbel at the onset of flowering which steadily increases until 50 or more florets open in one day at full bloom. Umbels are usually in bloom for a period of 30 days as the small flowers continue to open over a 2-3 week period. Individual *Allium* flowers are protandrous – the anthers dehisce before the stigma becomes receptive (Muller, 1883). When the anthers dehisce, all the pollen is shed within 24 to 36 hours, usually between 9 a.m. and 5 p.m. of the first day (McGregor, 1976). It has been found, under controlled conditions, that onion pollen remains viable for a period of 6 days (Mann & Woodbury, 1969). Temporal variation in pollen viability has also been recorded with pollen 2 to 3 times more viable when taken from flowers in the morning rather than in the evening (Nye *et al.*, 1971). However, Mann & Woodbury (1969) found no difference in viability of pollen grains sampled in the morning vs. in the afternoon.

Onion seeds are very small, black when ripe and born in silvery capsules (George, 1999). The size of the seeds vary between species and therefore also the number of seeds per gram of onion seed. Approximately 300 seeds per gram are generally recorded for onions (Brewster, 2008). The thousand-grain weight for onion seed is 3.6 g (George, 1999). Normally, the seed yield from F1 hybrids is lower (50-100 kg/ha) than from open pollinated crops (500-1000 kg/ha) with an optimum yield of 2000 kg/ha under ideal conditions (George, 1999).

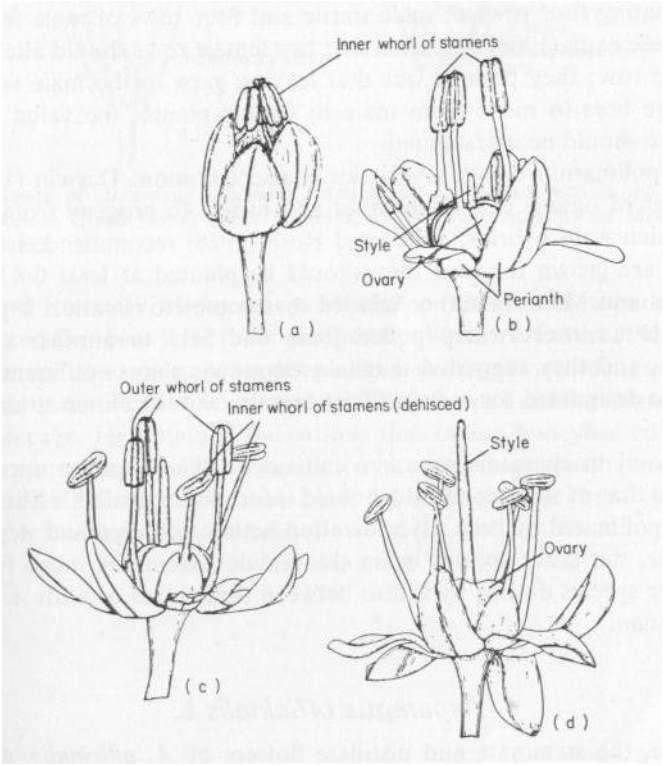


Figure 1.1: Opening stages in onion florets. a) perianth expanding and inner whorl of stamens elongating; b) inner whorl of stamens about to dehisce; c) inner whorl has dehisced and outer whorl is elongating; d) both whorls have dehisced. (from Jones & Rosa (1928)).

Onion flowers produce nectar which accumulates in three cups between the lower ovary walls and the inner whorl of stamens (Brewster, 2008). Onion nectar is usually highly concentrated with sugar concentrations exceeding 40% (Free, 1993b; Hagler *et al.*, 1990). Sugar concentrations were also found to differ considerably between hybrid parental lines (Silva & Dean, 2000). The sugar composition of onion nectar predominantly consist of relatively constant percentages of fructose and glucose between parental lines with almost undetectable levels of sucrose (Silva & Dean, 2000).

Onion flowers are often unattractive to honeybees (Gary et al., 1977, 1972), possibly due to the high potassium ion content (Waller *et al.,* 1972; Hagler, 1990) or the high sugar concentration in the nectar (Lederhouse *et al.,* 1972; Waters, 1972). Relatively large numbers of hives are required per hectare of onion seed crop and between 5 (open pollinated varieties) and 12 (hybrid varieties) hives are recommended to pollinate one hectare of onion crop (McGregor, 1976; Johannsmeier, 2001). This is in contrast to other vegetable seed crops dependent on managed honeybee hives, which generally only utilise an average of 2.4 hives

per hectare in South Africa (carrot: 2-8 hives/ha; pumpkin: 0.2-4 hives/ha; pepper and tomato: 1-2 hives/ha; brassicas: 2-5 hives/ha) (Johannsmeier, 2001). However, the attractiveness of flowering hybrid onion crops to honeybees has not been found to be a problem in some parts of the world. Honeybee visitation has been found to be sufficient in Poland (Kordakov, 1956; Woyke, 1981). This is in contrast with observations made in North America (Mayer and Lunden, 2001). Despite the general avoidance that the honeybee has for onion flowers, it has been found to be an abundant and important forager on onion crops (Witter & Blochtein, 2003; Howlett *et al.,* 2005).

In addition, the nectaries of *Allium* flowers are shallow and easily accessible to many types of pollinating insects, giving rise to a generalized entomophilous pollination syndrome (Brewster, 2008). A diversity of insect visitors to onion seed crops have been identified by several authors in various parts of the world (Caron *et al.*, 1975; Howlett *et al.*, 2005; Lederhouse *et al.*, 1968; Saeed *et al.*, 2008; Sajjad *et al.*, 2008; Williams & Free, 1974; Witter & Blochtein, 2003). Diptera and Hymenoptera species are often the most frequent visitors recorded on onion umbels (Free, 1993b; Williams & Free, 1974; Witter & Blochtein, 2003; Howlett *et al.*, 2005; Saeed *et al.*, 2008; Sajjad *et al.*, 2008). Bees have been found to be the most abundant native pollinators within onion seed crops in some studies (Parker, 1982; Witter & Blochtein, 2003; Howlett *et al.,* 2005), but flies were the more diverse group in other cases (Williams & Free, 1974; Saeed *et al.,* 2008; Sajjad *et al.,* 2008).

In addition, several authors have investigated the efficiency of pollinator taxa other than honeybees in pollinating onion flowers (Moffett, 1965; Walsh, 1965; Williams & Free, 1974; Parker, 1982; Currah & Ockendon, 1983, 1984; Schittenhelm *et al.,* 1997; Witter & Blochtein, 2003; Saeed *et al.,* 2008) and the role pollinator diversity may play in providing pollination as an ecosystem service to onion seed crops. Parker (1982) found that *Halictus farinosus* Smith (Hymenoptera: Halictidae), a native bee in North America, was the most abundant and also the most efficient pollinator in an experimental onion crop with male-fertile and male-sterile cultivars and supplemented with one managed hive. Flowers visited by honeybees aborted more seed than flowers visited by *Halictus* bees, and seed abortion rates were higher in self-pollinated flowers (Parker, 1982).

Saeed *et al.* (2008) assessed the pollination potential of true flies and native bees in Pakistan on hybrid seed onion crops and found that bees showed greater pollination effectiveness than flies. However, they did not report the presence of any managed honeybee

hives near the crop. In turn, Currah & Ockendon (1983, 1984) found no difference in pollination efficiency between honeybees and three species of blowflies in controlled experiments using large cages in terms of seed set and crossing level between two onion cultivars. In contrast, Walsh (1965) and Moffett (1965) found that houseflies were less efficient than honeybees in pollinating onion flowers in cages.

The bodies of honeybees have been found to carry more onion pollen than other insect visitors foraging on onion flowers (Kendall & Solomon, 1970; Free & Williams, 1972). However, Parker & Hatley (1979) assessed the viability of pollen grains on the bodies of pollinator insects and found that pollen on the bodies of honeybees were the least viable in onion pollination. Parker (1982) concluded that native bees could be and were often more efficient pollinators of onions than honey bees.

Many authors found more honeybees on male-fertile rows than on male-sterile rows in onion seed crop fields (Williams & Free, 1974; McGregor, 1976; Woyke, 1981; Parker, 1982; Mayer & Lunden, 2001). Only nectar collecting bees move freely between the two lines while pollen collecting bees tend to concentrate their activity on the male-fertile lines (McGregor, 1976). However, Williams & Free (1974) found that onion flowers were not favoured by honeybees as a source of pollen but that most foragers were nectar-gatherers. This does not explain the large abundance of foraging bees on male-fertile rows. Rather, it is more likely that the greater attractiveness of male-fertile onion flowers could be ascribed to the greater sugar concentration of the nectar (Lederhouse *et al.,* 1972) or their greater nectar production.

The only solution to the unattractiveness of onion flowers to honeybees thus far has been to stock onion hybrid seed crops excessively with honeybees. Strategies to make onion hybrid seed crops more attractive to honeybees have been investigated by various authors, which include testing aromatic attractants like Citral, Geraniol and anise, preconditioning honeybees with onion flower scent compounds and testing honeybee response to simulated onion nectars containing various sugar and potassium concentrations (Woyke, 1981; Hagler, 1990; Silva *et al.,* 2003).

Behavioural (competitive) interactions between honeybees and other pollinators in crop fields might be an important consideration when assessing the efficiency of wild and managed pollinators. Greenleaf & Kremen (2006) found in their investigation of the importance of behavioural interactions between honeybees and wild bees in field crops of sunflower hybrid seed that these interactions increased pollination up to 5-fold in some plots. These

interactions caused honeybees to move more readily from male-fertile to male-sterile lines, thus increasing visitation and pollen transfer rates. They highlighted the importance of conservation of natural habitat at a landscape scale and the implementation of pollinator friendly crop management in order to increase the production of hybrid sunflower by increasing the frequency of these types of interactions.

THE SOUTH AFRICAN SEED INDUSTRY

The seed industry in South Africa is well established and essentially run by the private sector with an estimated annual turnover of about USD 145 to USD 180 million (Van der Walt, 1999, 2002). The South African National Seed Organization (SANSOR) was established in 1989 as a uniting and permanent secretariat of the seed industry in South Africa and represents about 70 seed companies as well as about 500 distributing agencies (Van der Walt, 1999). Horticultural seed production (vegetable seeds) accounted for 21% of the South African seed market in 2000 (Van der Walt, 2002). Major vegetable seeds produced in South Africa include tomato, onion, brassicas, peppers, cucurbits, carrots, garden beans and sweet corn (Van der Walt, 2002). The estimated value of the domestic onion seed market in 2000 was USD 2.7 million (Van der Walt, 2002). This figure increased to about USD 21.8 million in the 2010/2011 financial book year (www.sansor.org). F_1 hybrid vegetable varieties are mainly imported from international companies while the country is particularly self-sufficient in terms of the ownership of plant breeders' rights and varieties of most other crop species (Van der Walt, 2002). By the end of 2000, South Africa owned 40% of the total number of plant breeders' rights registered globally, followed by the USA with 15% (Van der Walt, 2002).

Onion hybrid seed had been produced in South Africa since after World War II, since the establishment of hybrid onion cultivars, and was mainly undertaken by women (Johan Bekker pers. comm.). Today, onion hybrid seed production is a well-established farming practice undertaken by commercial seed growers (farmers), mainly in the Klein Karoo and areas in the southern Karoo in the Western Cape Province. These areas suit the climatic requirements for onion seed production which include low humidity and mild cool temperatures during the initial growth phase, followed by increased temperatures later on to induce flowering (Shanmugasundaram, 1998). Both these areas are within the highly diverse and endemic

Succulent Karoo biome. A total of 116 tons of onion hybrid seed were produced in South Africa in the 2010/2011 financial book year (www.sansor.org). Strict quality controls and regulations apply to ensure that seed growers produce the required quantity as well as quality of onion hybrid seed. Since 1990, government support to South African commercial farmers in the form of financial assistance and ensuring stable producer prices, gradually declined until today where it is almost completely non-existent (Van der Walt, 1999, 2002). Therefore strong incentives prevail to produce successful crops.

THE STUDY AREA AND ITS POLLINATOR COMMUNITY

The Klein Karoo is an oblong region of about 23 500 km^2 (Vlok & Schutte-Vlok, 2010), representing an extended valley that stretches from east to west between two mountain ranges parallel to the South African south coast, the Langeberg-Outeniqua mountain range in the south and the Witteberg-Swartberg mountain range in the north (refer to Figure A.1 in Appendix A). The mountain slopes are characterized by nutrient-poor sandstone soils hosting Fynbos vegetation while the low-lying areas generally have nutrient-rich loamy to clayey soils hosting Succulent Karoo vegetation types (Vlok & Schutte-Vlok, 2010). Three biomes meet in the Klein Karoo namely the Fynbos, Succulent Karoo and Subtropical Thicket biomes (Mucina & Rutherford, 2006). The Fynbos and Succulent Karoo biomes are recognised as global biodiversity hotspots (Myers *et al.,* 2000). The succulent Karoo vegetation are characterized by open to sparse dwarf (up to 1 m tall) shrubland dominated by stem and leaf succulents and some fine-leaved evergreen shrubs (Hilton-Tailor & Le Roux, 1989).

The Klein Karoo has a semi-arid climate which is dominated by orographic rainfall gradients and rain shadow effects, with the mountain ranges receiving above 1 000 mm of rain annually, while the low-lying central valley receives 100-300 mm per year (Le Maitre *et al.*, 2009). The rainfall season varies from west to east throughout the Klein Karoo, whereby the western region (Montagu) receives winter rainfall which progressively shifts to summer rainfall events received by the east (Willowmore) (Le Maitre *et al.,* 2009a). The mean daily temperature ranges between maximum temperatures above 30 °C in February to about 20-22 °C in August (Le Maitre *et al.,* 2009a).

The southern Karoo is also a semi-arid region stretching northward from the Witteberg-Swartberg mountain range (CEPF, 2003) and also hosts succulent Karoo vegetation (Tainton,

1999). The Karoo has shallow, weakly developed alkaline soils (Cowling, 1986) and are not suitable for cultivation, except for alluvial terraces which had been utilized extensively (Dean & Milton, 1995). As with most arid and semi-arid regions, the southern Karoo are characterized by extreme temperatures and unpredictable and highly variable rainfall of about 170 mm annually (Cowling, 1986; Dean & Milton, 1995). Detailed descriptions of the ecology, geography and climatology of the Little Karoo and southern Karoo are given by Cowling, 1986; Cowling & Roux, 1987; Cowling *et al.*, 1997; Cowling *et al.*, 1986; Dean & Milton, 1999 and Vlok & Schutte-Vlok, 2010.

Intensive livestock farming and overgrazing is considered to be the single largest cause of degraded succulent Karoo vegetation in the low-lying valleys of the Klein Karoo and southern Karoo (Milton *et al.,* 1994; Cupido, 2005). Ostrich farming is a dominant farming practice in the Klein Karoo and commenced between 1857 and 1860 (Beyleveld, 1967). Large concentrations of ostriches are confined to small camps where trampling causes severe transformation and degradation of the vegetation and soil (Cupido, 2005; Hoffman *et al.,* 1999). A living soil crust of cyanobacteria, lichens and mosses on the soils of the low-lying areas of the Klein Karoo plays an essential role in facilitating nutrient cycling, water infiltration and the prevention of soil erosion (Vlok & Schutte-Vlok, 2010). This biocrust is exceptionally resilient to drought conditions but is sensitive and vulnerable to trampling and overgrazing by livestock (Vlok & Schutte-Vlok, 2010).

Crop cultivation in the Klein Karoo and southern Karoo are confined largely to alluvial habitats providing access to richer soils and irrigation water (Dean & Milton, 1995; Thompson *et al.,* 2005). Crop production accounts for about 10% of the total loss of habitat in the Klein Karoo (Dean & Milton, 1995; Thompson *et al.,* 2005) (Figure 1.2). Lucerne (*Medicago sativa* L.) is the dominant agricultural crop grown in the Klein Karoo, grown mainly to supply fodder for the ostrich farming industry (Cupido, 2005). However, vegetable seed production also provides a valuable source of income to farmers in the region. Many farmers in the region are involved in a diversity of agricultural practices and other economic activities such as tourism in an effort to attain economic resilience (Le Maitre *et al.,* 2009b).

a

b

Figure 1.2: Onion hybrid seed crop fields grown in the Klein Karoo (a) and southern Karoo (b). The predominant vegetation growth can be seen in the fore- and backgrounds, with green thorn trees lining the river beds.

Only 3.5% of the Succulent Karoo biome is formally protected (Driver *et al.,* 2003). Because of the high biodiversity value and endemism of the succulent Karoo vegetation and its status of being highly threatened, several initiatives have been launched to document the diversity and conservation status within the biome. Three of these are internationally funded

conservation programs aimed at identifying projects to enhance and implement conservation strategies within the region. They are C.A.P.E. (Cape Action Plan for People and the Environment), S.K.E.P. (Succulent Karoo Ecosystem Plan) and S.T.E.P. (Subtropical Thicket Ecosystem Program). In the Klein Karoo, the Gouritz Initiative (GI) was established in 2003 to coordinate conservation strategies, facilitate co-governance and meet the needs of stakeholders from diverse spheres within the region demarcated by the Gouritz River watershed and catchments areas (www.gouritz.com). The GI was launched under S.K.E.P. and C.A.P.E. and aimed to promote and establish a system of sustainable living landscapes through partnerships between existing nature reserves and private landowners within the specific region. Landowners are encouraged to adopt conservation conscious farming methods and to set aside portions of conservation-worthy land for conservation where possible. Recent developments within the GI comprise a transition to the Gouritz Cluster Biosphere Reserve which will be recognized as a UNESCO Biosphere Reserve. Pollinators and their conservation should benefit from such initiatives.

Historically, pollination biology research has mainly been focussed on the Fynbos flora with resent progress being made in documenting pollination systems in the Succulent Karoo and Grassland biomes (Johnson, 2004). However, the bulk of the work focussed mainly on evolutionary pollination studies, while little work has been done on applied pollination biology concerning conservation and agriculture in Africa, including South Africa (Rodger *et al.*, 2004). It is only recently that pollination studies in Africa and particularly in South Africa, started to focus on the pollinator community level (Rodger *et al.*, 2004).

The Succulent Karoo biome is characterized by mass flowering displays which reaches a climax near the West Coast and attracts large numbers of tourists (O'Farrell *et al.,* 2011). These floral displays are recognized as an ecosystem service and the estimated value of flower viewing tourism in the north-western areas of the succulent Karoo was calculated to be R18 million in 2009 (Le Maitre *et al.,* 2009b; O'Farrell *et al.,* 2011). Several researchers embarked on documenting the pollinator communities of the succulent Karoo, its interaction with the flowering plant communities and its response to environmental gradients and anthropogenic pressures (Gess, 1992, 2001; Gess & Gess, 1993, 2004a, 2004b; Struck, 1994a, 1994b; Manning & Goldblatt, 1996; Colville *et al.,* 2002; Johnson, 2004; Mayer, 2004; Mayer *et al.,* 2006). These pollinator communities deliver an ecosystem service to the flowering plants of the succulent Karoo. Flowering plants such as Mesembryanthemaceae, *Galenia*, *Lycium*,

Fabaceae, *Diospyros*, and *Acacia karroo* provide a valuable ecosystem service to commercial beekeepers by providing nectar and pollen resources to managed Cape honeybee colonies that in turn provide seasonal pollination services to orchards and vegetable seed crops in the Tanqua, southern Karoo and Klein Karoo (Le Maitre et al., 2009b).

Pollen wasps (Hymenoptera: Vespidae: Masarinae) are more species diverse in the Karoo than in any other part of the world and all of them are endemic to the region (Gess, 1996, 2001). South Africa is also a globally significant centre for bee diversity with a high degree of endemism (Whitehead, 1984; Eardley, 1996; Kuhlmann, 2009). The most important centres of endemism are located in the western arid winter-rainfall areas which include the Fynbos and Succulent Karoo biomes, and the eastern early to mid-summer rainfall areas which include the Grassland and Savanna biomes (Kuhlmann, 2009). The peak adaptive radiation of Money beetles (Coleoptera: Scarabaeidae: Hopliini) occurred in the Succulent Karoo resulting in an abundance of species (Picker & Midgley, 1996). They are important pollinators of Asteraceae flowers (Picker & Midgley, 1996). Other pollinator groups showing high diversity and endemism in the Succulent Karoo include bee-flies (Bombyliidae) (Hesse, 1938; Struck, 1994a) and tangle-veined flies (Nemestrinidae) (Manning & Goldblatt, 1996). However, thus far, little work has aimed to determine pollinator limitation in Succulent Karoo vegetation (Cowling *et al.,* 1999). Mayer (2004) found that fruit set of Aizoaseae species were compromised on overgrazed land, while insect diversity and abundance were partially reduced under heavy grazing, especially where vegetation cover was low and dominated by unpalatable species.

Gess & Gess (1993) evaluated the species representation and diversity of aculeate wasps and bees in the semi-arid regions of southern Africa in the light of increasing land utilization. Solitary bees and aculeate wasps are known to be habitat specialists and show a high level of beta diversity, leaving them vulnerable to changes to their habitat as they are not readily adaptable to change (Gess, 2001). Because of the semi-arid to arid climate of the Karoo, the most suitable areas for large-scale cultivation is along water courses; also the sites where a wide range of bees and aculeate wasps make their nests because of the availability of water (Gess, 2001). Thus farming practices in arid and semi-arid areas pose a real threat to pollinator insects. Gess (2001) identified several other factors that have an adverse effect on the diversity of pollinators (especially solitary bees and aculeate wasps) in the Karoo. The factors that are related to crop cultivation include the extensive replacement of natural

vegetation by crops and cultivated pastures, the use of insecticides for crop protection, the spread of invasive exotic plant species, bush cutting, large-scale water canalling and impoundment and the intensive removal of dry wood. It is important to generate an understanding of pollinator requirements and to consider these requirements in land management practices (Gess, 2002).

RESEARCH OBJECTIVES

In similarity to most other pollinator-dependent commercial agricultural crops produced worldwide, the honeybee is generally accepted to be the main pollinator of onion hybrid seed crops in South Africa. The Cape honeybee, *Apis mellifera capensis* Esch., is indigenous to the Western Cape province where onion hybrid seed is produced and is used for honey production and managed pollination services. *A.m. capensis* is also a dominant and important element of the natural pollinator assemblages of Western Cape ecosystems. As one of the projects funded by the Global Pollination Project, this research fulfils the first of the four elements of the IPI Plan of Action, which is to extend our knowledge base of PES through assessment (refer to p.7), and does this by quantifying PES delivered to onion hybrid seed crops in South Africa. The research aims to answer four main questions regarding the importance of PES to onion hybrid seed crops in S.A. These include:

1) *Does natural habitat availability determine anthophile diversity within onion hybrid seed crops?*

Here the aim is to relate the richness and abundance of insect flower-visitors found within onion hybrid seed crops during peak flowering to the amount of natural habitat within a buffer zone of 500 m from the crop periphery which is assumed to be source habitats of pollinator insects. Flower-visiting insects were sampled extensively within blooming onion crops using coloured pan traps as a passive sampling method. This diversity was also related to farm management practices such as the methods used to irrigate crop fields to assess whether they have any significant effects on anthophile diversity.

2) *Which insect visitors are likely to be the main pollinators of onion hybrid seed crops in South Africa?*

Direct observation data and hand-sampled specimens aid in identifying the true and frequent insect visitors to hybrid onion umbels. Pollen loads carried on the bodies of frequent

umbel visitors are measured and compared between species and between male-fertile and male-sterile rows to determine whether the species is indeed a hybrid onion pollen vector and hence its probability of being a pollinator of the crop.

3) *Are onion hybrid seed yields dependent on pollinator diversity?*

Umbel seed yield is used as an indication of pollination deficits and is compared between crops with varying degrees of pollinator diversity and also between crops across a gradient of available natural habitat. The visitation frequency of honeybees and non-*Apis* visitors, and the varying stocking densities of managed honeybee colonies are also considered as important factors in the analysis.

4) *Are honeybee foraging patterns altered by other insect visitors foraging on the umbels and what are the factors that cause honeybees to forage discriminately between parental lines?*

Greenleaf & Kremen (2006a) reported that inter-specific interactions between honeybees and non-*Apis* bees improved honeybees' pollination of hybrid sunflower five-fold. When honeybees encountered other bee species on male-fertile sunflower heads they were 20% more likely to move to male-sterile rows as opposed to when another honeybee was encountered, then the probability was only 7% to move to male-sterile sunflower heads. After Greenleaf & Kremen (2006a), nectar-foraging honeybees were followed on male-fertile umbels to record their behaviour after encounters with non-*Apis* flower visitors. Hence to determine whether non-*Apis* visitors possibly contribute indirectly to hybrid onion pollination in South Africa. Onion flower nectar and umbel scent were measured and compared between parental lines as factors that could play a role in the successful pollination of onion hybrid seed crops.

A successful onion hybrid seed crop is the result of considerable effort and input during several production phases, stretching over a period of about two years. But in the end, a successful yield is for the most part, delivered to and ultimately depend on successful pollination.

REFERENCES

Aizen, M.A., Feinsinger, P., 1994. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine "Chaco Serrano". *Ecological Applications* 4, 378–392.

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.-M., 2008. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Current Biology* 18, 1572–1575.

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.-M., 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany* 103, 1579–1588.

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S.L., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D.W., Eugene, C., Kennedy, K., Kevan, P.G., Koopowitz, H., Medellin, R., Nabhan, G.P., Pavlik, B., Tepedino, V.J., Torchio, P.F., 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* 12, 8–17.

Allsopp, M.H., 2009. Bee disease threatens South African apiculture, *Plant Protection News*, No. 79, Agricultural Research Council: Plant Protection Research Institute.

Altieri, M.A., 1995. *Agroecology: the science of sustainable agriculture*. Westview Press, Boulder, CO.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. *Agriculcture Ecosystems and Environment* 74, 19–31.

Bernhardt, P., 2000. Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. *Plant Systematics and Evolution* 222, 293–320.

Beyleveld, A.J., 1967. Ostrich farming in South Africa. *Agrekon* 6, 14–16.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313, 351–354.

Blanche, K.R., Cunningham, S.A., 2005. Rainforest provides pollinating beetles for Atemoya crops. *Journal of Economic Entomology* 98, 1193–1201.

Blanche, K.R., Ludwig, J.A., Cunningham, S.A., 2006. Proximity to rainforest enhances pollination and fruit set in orchards. Journal of Applied Ecology. 43, 1182–1187.

Bohart, G.E., Nye, W.P., Hawthorn, L.R., 1970. Onion pollination as affected by different levels of pollinator activity. *Utah Agricultural Experiment Station Bulletin*, No. 482, 66.

Boyd, J., Banzhaf, S., 2007. What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics* 63, 616–626.

Brewster, J.L., 2008. *Onions and other vegetable Alliums, 2nd Edition*. CAB International, Oxon.

Brosi, B.J., Daily, G.C., Shih, T.M., Oviedo, F., DurÃ¡n, G., 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology* 45, 773–783.

Buckmann, S.L., Nabhan, G.P., 1996. *The forgotten pollinators*. Island Press, Washington, DC.

Buscher, B.E., 2008. Conservation, neoliberalism, and social science: a critical reflection on the SCB 2007 annual meeting in South Africa. *Conservation Biology* 22, 229–231.

Caron, D.M., Lederhouse, R.C., Morse, R.A., 1975. Insect pollinators of onion in New York State. *Hortscience* 10, 273–274.

CEPF, 2003. *Ecosystem profile: The Succulent Karoo hotspot, Namibia and South Africa, Africa*. Critical Ecosystem Partnership Fund.

Chacoff, N.P., Aizen, M.A., 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. *Journal of Applied Ecology*. 43, 18–27.

Clark, W.C., Dickson, N.M., 2003. Sustainability science: the emerging research program. *Proceedings of the National Academy of Sciences* 100, 8059–8061.

Colville, J.F., Picker, M.D., Cowling, R.M., 2002. Species turnover of monkey beetles (Scarabaeidae: Hopliini) along environmental and disturbance gradients in the Namaqualand region of the succulent Karoo, South Africa. *Biodiversity and Conservation* 11, 243–264.

Costanza, R., D'Arge, R., de Groot, R.S., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V, Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260.

Costanza, R., D'Arge, R., de Groot, R.S., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V, Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1998. The value of the world's ecosystem services and natural capital. *Ecological Economics* 25, 3–15.

Cowling, R.M., 1986. *A description of the Karoo Biome Project*. South African National Scientific Programmes Report No. 122. Pretoria.

Cowling, R.M., Egoh, B., Knight, A.T., Farrell, P.J.O., Reyers, B., Rouget, M., Roux, D.J., Welz, A., Wilhelm-Rechman, A., O'Farrell, P.J., 2008. An operational model for mainstreaming ecosystem services for implementation. *Proceedings of the National Academy of Sciences* 105, 9483–9488.

Cowling, R.M., Esler, K.J., Rundel, P.W., 1999. Namaqualand, South Africa – an overview of a unique winter-rainfall desert ecosystem. *Plant Ecology* 142, 3–21.

Cowling, R.M., Richardson, D.M., Pierce, S.M. (Eds.), 1997. *Vegetation of southern Africa*. Cambridge University Press, Cambridge.

Cowling, R.M., Roux, P.W., 1987. *The karoo biome: a preliminary synthesis Part 2 - vegetation and history*. South African National Scientific Programmes Report, No. 142, Pretoria.

Cowling, R.M., Roux, P.W., Pieterse, A.I.H., 1986. *The karoo biome: a preliminary synthesis. Part 1 - physical environment*. South African National Scientific Programmes Report, No. 142, Pretoria.

Cremo, M.A., Goswani, M., 1995. *Divine nature: a spiritual perspective on the environmental crisis*. Bhaktivedanta Book Trust International.

Cupido, C.F., 2005. *Assessment of veld utilisation practices and veld condition in the Little Karoo*. MSc thesis, Stellenbosch University.

Currah, L., Ockendon, D.J., 1983. Onion pollination by blowflies and honeybees in large cages. *Annals of Applied Biology* 103, 497–506.

Currah, L., Ockendon, D.J., 1984. Pollination activity by blowflies and honeybees on onions in breeders' cages. *Annals of Applied Biology* 105, 167–176.

Daily, G.C., 1997. *Nature's services: societal dependence on natural ecosystems*. Island Press, Washington, D.C.

Daily, G.C., 2000. Management objectives for the protection of ecosystem services. *Environmental Science & Policy* 3, 333–339.

Daily, G.C., Matson, P.A., 2008. Ecosystem services: From theory to implementation. *Proceedings of the National Academy of Sciences* 105, 9455–9456.

De Groot, R.S., Wilson, M.A., Boumans, R.M.J., 2002. A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological Economics* 41, 393– 408.

De Marco, P., Coelho, F.M., 2004. Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity and Conservation* 13, 1245–1255.

De Ruijter, A., 2002. Pollinator diversity and sustainable agriculture in the Netherlands, in: Kevan, P., Imperatriz-Fonseca, V.L. (Eds.), *Pollinating bees: the conservation link between agriculture and nature*. Ministry of Environment, Brasilia, pp. 67–70.

Dean, W.R.J., Milton, S.J., 1995. Plant and invertebrate assemblages on old fields in the arid southern Karoo, South Africa. *African Journal of Ecology* 33, 1–13.

Dean, W.R.J., Milton, S.J., 1999. *The Karoo: ecological patterns and processes*. Cambridge University Press, Cambridge.

Donaldson, J.S., Nänni, I., Zachariades, C., Kemper, J., 2002. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conservation Biology* 16, 1267–1276.

Driver, A., Desmet, P.G., Rouget, M., Cowling, R.M., Maze, K., 2003. *Succulent Karoo ecosystem plan*. Biodiversity component technical report, Cape Conservation Unit Report No CCU 1/03. Kirstenbosch, Cape Town.

Eardley, C.D., 1996. The genus *Scrapter* Lepeletier & Serville (Hymenoptera: Colletidae). *African Entomology* 4, 37–92.

Ehrlich, P.R., Raven, P.H., 1964. Butterflies and plants: a study in coevolution. *Evolution* 18, 586–608.

Esty, D., Levy, M.A., Srebotnjak, T., de Sherbinin, A., 2005. 2005 *Environmental sustainability index: benchmarking national environmental stewardship*. Yale Center for Environmental Law and Policy, New Haven.

Fisher, B., Turner, R.K., 2008. Ecosystem services: Classification for valuation. *Biological Conservation* 141, 1167–1169.

Fisher, B., Turner, R.K., Morling, P., 2009. Defining and classifying ecosystem services for decision making. *Ecological Economics* 68, 643–653.

Free, J.B., 1993a. *Insect pollination of crops*. Academic Press, London, UK.

Free, J.B., 1993b. Alliaceae, in: *Insect Pollination of Crops*. Academic Press, London, UK, pp. 115–121.

Free, J.B., Williams, I.H., 1972. The transport of pollen on the body hairs of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus* spp. L.). *Journal of Applied Ecology* 9, 609–615.

Gallai, N., Salles, J., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* 68, 810–821.

Gary, N.E., Witherell, P.C., Lorenzen, K., Marston, J.M., 1977. The interfield distribution of honey bees foraging on carrots, onions and safflower. *Environmental Entomology* 6, 637–640.

Gary, N.E., Witherell, P.C., Marston, J.M., 1972. Foraging range and distribution of honey bees used for carrot and onion pollination. *Environmental Entomology* 1, 71–78.

George, R.A.T., 1999. *Vegetable seed production, 2nd Edition*. CAB Interantional Publishing, New York.

Gess, F.W., Gess, S.K., 1993. Effects of increasing land utilization on species representation and diversity of aculeate wasps and bees in the semi-arid areas of southern Africa., in: La Salle, J., Gauld, I.D. (Eds.), *Hymenoptera and Biodiversity*. CAB International, Wallingford, England, pp. 83–113.

Gess, S.K., 1992. Biogeography of the masarine wasps (Hymenoptera: Vespidae: Masarinae), with particular emphasis on the southern African taxa and on correlations between masarine and forage plant distributions . *Journal of Biogeography* 19, 491–503.

Gess, S.K., 1996. *The pollen wasps: ecology and natural history of the Masarinae*. Harvard University Press, Cambridge.

Gess, S.K., 2001. *The Karoo, its insect pollinators and the perils which they face*. International Pollinator Initiative (IPI) Case Study. (www.ecoport.org)

Gess, S.K., Gess, F.W., 2004a. Distributions of flower associations of pollen wasps (Vespidae: Masarinae) in southern Africa. *Journal of Arid Environments* 57, 17–44.

Gess, S.K., Gess, F.W., 2004b. A comparative overview of flower visiting by non-Apis bees in the semi-arid to arid areas of southern Africa. *Journal of the Kansas Entomological Society* 77, 602–618.

Gill, R.J., Ramos-Rodriguez, O., Raine, N.E., 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491, 105–8.

Goldman, R.L., Tallis, H., Kareiva, P., Daily, G.C., Goldman, L., Dailyt, G.C., 2008. Field evidence that ecosystem service projects support biodiversity and diversify options. *Proceedings of the National Academy of Sciences* 105, 9445–9448.

Greenleaf, S.S., Kremen, C., 2006a. Wild bees enhance honey bees ' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences* 103, 13890–13895.

Greenleaf, S.S., Kremen, C., 2006b. Wild bee species increase tomato production and respond differently to surrounding land use in North California. *Biological Conservation* 133, 81–87.

Hagler, J.R., 1990. Honey bee (*Apis mellifera* L.) response to simulated onion nectars containing variable sugar and potassium concentrations. *Apidologie* 21, 115–121.

Hagler, J.R., Cohen, A.C., Loper, G.M., 1990. Production and composition of onion nectar and honey bee (Hymenoptera: Apidae) foraging activity in Arizona. *Environmental Entomology* 19, 327–331.

Harris, L.F., Johnson, S.D., 2004. The consequences of habitat fragmentation for plant–pollinator mutualisms. *International Journal of Tropical Insect Science* 24, 29–43.

Heal, G., 2000. Valuing ecosystem services. *Ecosystems* 3, 24–30.

Heard, T.A., Exley, E., 1994. Diversity, abundance and distribution of insect visitors to macadamia flowers. *Environmental Entomology* 23, 91–100.

Hesse, A.J., 1938. A revision of the Bombyliidae (Diptera) of southern Africa. Part 1. A*nnals of the South African Museum* 34, 1–1053.

Hilton-Tailor, C., Le Roux, A., 1989. Conservation status of the Fynbos and Karoo biomes, in: Huntley, B.J. (Ed.), *Biotic Diversity in southern Africa*. Oxford University Press, Cape Town, pp. 202–223.

Hoffman, M.T., Cousins, B., Meyer, T., Petersen, A., Hendricks, H., 1999. Historical and contemporary land use and the desertification of the Karoo, in: Dean, W.R.J., Milton, S.J. (Eds.), *The Karoo: Ecological Patterns and Processes*. Cambridge University Press, Cambridge, pp. 257–273.

Howlett, B.G., Donovan, B.J., McCallum, J.A., Newstrom, L.E., Teulon, D.A.J., 2005. Between and within field variability of New Zealand indigenous flower visitors to onions. *New Zealand Plant Protection* 58, 213–218.

Johannsmeier, M.F., 2001. *Beekeeping in South Africa, 3rd Edition*. Agricultural Research Council-Plant Protection Research Institute, Handbook No. 14, Pretoria.

Johannsmeier, M.F., 2005. *Beeplants of the south-Western Cape*. Agicultural Research Council-Plant Protection Research Institute, Handbook No. 17., Pretoria.

Johnson, S.D., 2004. An overview of plant–pollinator relationships in southern Africa. *International Journal of Tropical Insect Science* 24, 45–54.

Jones, H.A., Clarke, A.E., 1943. Inheritance of male sterility in the onion and the production of hybrid seed. *Proceedings of the American Society for Horticultural Science* 43, 189–194.

Jones, H.A., Mann, L.M., 1963. *Onions and their allies, botany, cultivation and utilization*. Leongard Hill, London.

Jones, H.A., Rosa, J.T., 1928. *Truck crop plants, 1st Edition*. McGraw-Hill, New York.

Kendall, D.A., Solomon, M.E., 1970. *Insect pollination of fruit trees*, Republication of the Agriculture Horticultural Research Station, University of Bristol.

Klein, A.-M., Steffan-Dewenter, I., Tscharntke, T., 2003a. Pollination of Coffea canephora in relation to local and regional agroforestry management. Journal of Applied Ecology 40, 837– 845.

Klein, A.-M., Steffan-Dewenter, I., Tscharntke, T., 2003b. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society B: Biological Sciences* 270, 955–961.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274, 303–313.

Kluser, S., Peduzzi, P., 2007. *Global pollinator decline: a literature review*. UNEP/GRID, Geneva.

Kordakov, Z.M., 1956. Honeybees and pollination of seed plants of the common onion, in: Gubin, A.F. (Ed.), *Pollination of Agricultural Crops*. Gos. Izd-vo Selkhoz Litry, pp. 163–171.

Kosior, A., Celary, W., Olejniczak, P., Fijal, J., Król, W., Solarz, W., Plonka, P., 2007. The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. *Oryx* 41, 79–88.

Kremen, C., Williams, N.M., Bugg, R.L., Fray, J.P., Thorp, R.W., 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* 7, 1109–1119.

Kremen, C., Williams, N.M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences* 99, 16812–16816.

Kuhlmann, M., 2009. Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in southern Africa. *South African Journal of Botany* 75, 726–738.

Lamm, R.D., 2006. The culture of growth and the culture of limits. *Conservation Biology* 20, 269–271.

Le Maitre, D.C., Colvin, C., Maherry, A., 2009a. Water resources in the Klein Karoo: the challenge of sustainable development in a water-scarce area. *South African Journal of Science* 105, 39–48.

Le Maitre, D.C., O'Farrell, P.J., Milton, S.J., Atkinson, D., De Lange, W.J., Egoh, B., Reyers, B., Colvin, C., Maherry, A., Blignaut, J.N., 2009b. *Assessment and evaluation of ecosystem services in the Succulent Karoo biome*. Council for Scientific and Industrial Research, Pretoria.

Leadley, P., Pereira, H.M., Alkemade, R., Fernandez-Manjarres, J.F., Proenca, V., Scharlemann, J.P.W., Walpole, M.J., 2010. *Biodiversity scenarios: projections of 21st century - change in biodiversity and associated ecosystem services*. Secretariat of the Convention on Biological Diversity, Montreal.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1968. Onion pollination in New York. *New York's Food Life Sciences Bulletin* No. 1, 8–9.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1972. Distribution and behavior of honey bees on onion. *Environmental Entomology* 1, 127–129.

Mann, L.P., Woodbury, G.W., 1969. The effect of flower age, time of day and variety on pollen germination of onion, *Allium cepa* L. *Journal of the American Society of Horticultural Science* 94, 102–104.

Manning, J.C., Goldblatt, P., 1996. The *Prosoeca peringueyi* (Diptera: Nemestrinidae) pollination guild in southern Africa: long-tongued flies and their tubular flowers. *Annals of the Missouri Botanical Garden* 83, 67–86.

Mayer, C., 2004. Pollination services under different grazing intensities. *International Journal of Tropical Insect Science* 24, 95–103.

Mayer, C., Soka, G., Picker, M.D., 2006. The importance of monkey beetle (Scarabaeidae: Hopliini) pollination for Aizoaceae and Asteraceae in grazed and ungrazed areas at Paulshoek, Succulent Karoo, South Africa. *Journal of Insect Conservation*. 10, 323–333.

Mayer, D.F., Lunden, J.D., 2001. Honey bee management and wild bees for pollination of hybrid onion seed. *Acta Horticulturae*. 561, 275–278.

McGregor, S.E., 1976. Common vegetables for seed and fruit, in: McGregor, S.E. (Ed.), *Insect Pollination of Cultivated Crop Plants*. United States Department of Agriculture, Washington.

Meine, C., Soulé, M., Noss, R.F., Soule, M., 2006. "A mission-driven discipline": the growth of conservation biology. *Conservation Biology* 20, 631–651.

Millenium Ecosystem Assessment, 2005a. *Ecosystems and human well-being: biodiversity synthesis*. Island Press, Washington, DC.

Millenium Ecosystem Assessment, 2005b. *Ecosystems and human well-being: current state and trends*. Island Press, Washington, DC.

Millenium Ecosystem Assessment, 2005c. Biodiversity regulation of ecosystem services, in: *Ecosystems and human well-being: current state and trends*. Island Press, Washington, DC., pp. 297–329.

Milton, S.J., Dean, W.R.J., Plessis, M.A., Siegfried, W.R., 1994. A conceptual model of arid rangeland degradation. *Bioscience* 44, 70–76.

Moffett, J.O., 1965. Pollinating experimental onion varieties. *American Bee Journal* 105, 378.

Morandin, L.A., Winston, M.L., 2005. Wild bee abundance and seed production in conventional, organic, and genetically modified Canola. *Ecological Applications* 15, 871–881.

Mucina, L., Rutherford, M.C. (Eds.), 2006. *The vegetation of South Africa, Lesotho and Swaziland, Strelitzia 19*. South African National Biodiversity Institute, Pretoria.

Muller, H., 1883. *The fertilisation of flowers*. Macmillan, London.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., de Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.

Nye, W.P., Waller, G.D., Waters, N., 1971. Factors affecting pollination of onions in Idaho during 1969. Journal of the American Society of Horticultural Science 96, 330–332.

O'Farrell, P.J., De Lange, W.J., Le Maitre, D.C., Reyers, B., Blignaut, J.N., Milton, S.J., Atkinson, D., Egoh, B., Maherry, A., Colvin, C., Cowling, R.M., 2011. The possibilities and pitfalls presented by a pragmatic approach to ecosystem service valuation in an arid biodiversity hotspot. *Journal of Arid Environments* 75, 612–623.

O'Toole, C., 1993. Diversity of native bees and agroecosystems, in: LaSalle, J., Gauld, I.D. (Eds.), *Hymenoptera and Biodiversity*. CAB International, Wallingford, UK.

Oldroyd, B.P., 2007. What's killing American honey bees? *PLoS Biology*. 5, e168.

Parker, F.D., 1982. Efficiency of bees in pollinating onion flowers. *Journal of the Kansas Entomological Society* 55, 171–176.

Parker, F.D., Hatley, C.L., 1979. Onion pollination: viability of onion pollen and pollen diversity on insect body hairs., in: *Proceedings of the Fourth International Symposium on Pollination*. Maryland agricultural experiment station special miscellaneous publication 1, pp. 201–206.

Pathak, C.S., 2000. Hybrid seed production in onion. *Journal of New Seeds* 1, 89–108.

Picker, M.D., Midgley, J.J., 1996. Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): flower and colour preferences. *African Entomology* 4, 7–14.

Pimm, S.L., Russell, G.J., Gittleman, J.L., Brooks, T.M., 1995. The future of biodiversity. *Science* 269, 347–350.

Rees, W.E., 1992. Ecological footprints and appropriated carrying capacity: what urban economics leaves out. *Environment and Urbanization* 4, 121–130.

Richards, K.W., 1993. Non-*Apis* bees as crop pollinators. *Revue Suisse Zoology* 100, 807–822.

Ricketts, T.H., 2004. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* 18, 1262–1271.

Ricketts, T.H., Daily, G.C., Ehrlich, P.R., Michener, C.D., 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences* 101, 12579–12582.

Rodger, J.G., Balkwill, K., Gemmill-Herren, B., 2004. African pollination studies: where are the gaps? *International Journal of Tropical Insect Science* 24, 5–28.

Saeed, S., Sajjad, A., Kwon, O., Kwon, Y.J., 2008. Fidelity of Hymenoptera and Diptera pollinators in onion (*Allium cepa* L.) pollination. *Entomological Research* 38, 276–280.

Sajjad, A., Saeed, S., Masood, A., 2008. Pollinator community of onion (*Allium cepa* L.) and its role in crop reproductive success. *Pakistan Journal of Zoology* 40, 451–456.

Schittenhelm, S., Gladis, T., Rao, V.R., 1997. Efficiency of various insects in germplasm regeneration of carrot, onion and turnip rape accessions. *Plant Breeding* 116, 369–375.

Shanmugasundaram, S., 1998. *Onion cultivation and seed production*. Shanhua, Tainan, AVRDC.

Shepherd, M., Buckmann, S.L., Vaughan, M., Hoffmann-Black, S., 2003. *Pollinator conservation handbook*. The Xerces Society, Oregon.

Silva, E.M., Dean, B.B., 2000. Effect of nectar composition and nectar concentration on honey bee (Hymenoptera: Apidae) visitations to hybrid onion flowers. *Journal of Economic Entomology* 93, 1216–1221.

Silva, E.M., Dean, B.B., Hiller, L.K., 2003. Honey Bee (Hymenoptera: Apidae) foraging in response to preconditioning with onion flower scent compounds. *Journal of Economic Entomology* 96, 1510–1513.

Steyn, T., 2009. Bysiekte in tot 85% van korwe. *Die Burg*er, 14 July, p.6.

Struck, M., 1994a. Flowers and their insect visitors in the arid winter rainfall region of southern Africa: observations on permanent plots. Insect visitation behaviour. *Journal of Arid Environments* 28, 51–74.

Struck, M., 1994b. Flowers and their insect visitors in the arid winter rainfall region of southern Africa: observations on permanent plots. Composition of the anthophilous insect fauna. *Journal of Arid Environments* 28, 45–50.

Tainton, N. (Ed.), 1999. *Veld management in South Africa*. University of Natal Press, Pietermaritzburg.

Thompson, M., Vlok, J., Cowling, R.M., Cundill, S.L., Mundau, N., 2005. *A land transformation map for the Little Karoo*. GeoterraImage, Pretoria.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven global environmental change. *Science* 292, 281–284.

Van der Walt, W.J., 1999. Changing face of South African seed trade. *Journal of New Seeds* 1, 81–97.

Van der Walt, W.J., 2002. A review of the South African seed industry. *Journal of New Seeds* 4, 57–70.

Vavilov, N.I., 1951. The origin, variation, immunity and breeding of cultivated plants. *Chronica Botanica* 13, 1–6.

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of Earth's ecosystems. *Science* 277, 494–499.

Vlok, J., Schutte-Vlok, A.L., 2010. *Plants of the Klein Karoo*. Umdaus Press, Pretoria.

Wackernagel, M., 1994. *Ecological footprint and appropriated carrying capacity: a tool for planning toward sustainability*. PhD thesis, The University of British Columbia.

Wallace, K.J., 2007. Classification of ecosystem services: Problems and solutions. *Biological Conservation* 139, 235–246.

Waller, G.D., Carpenter, E.W., Ziehl, O.A., 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. *Journal of the American Society of Horticultural Science* 97, 535–539.

Walsh, R.S., 1965. Pollination of onion plants by honeybees. *New Zealand Beekeeper* 27, 18–20.

Watanabe, M.E., 1994. Pollination worries rise as honey bees decline. *Science* 265, 1170.

Waters, N.D., 1972. Honeybee activity in blooming onion fields in Idaho. *American Bee Journal* 112, 218–219.

Whitehead, V.B., 1984. Distribution, biology and flower relationships of fideliid bees of southern Africa (Hymenoptera, Apoidea, Fideliidae). *South African Journal of Zoology* 19, 87–90.

Williams, I.H., Free, J.B., 1974. The pollination of onion (*Allium cepa* L.) to produce hybrid seed. *Journal of Applied Ecology* 11, 409–417.

Williams, N.M., Minckley, R.L., Silveira, F.A., 2001. Variation in native bee faunas and its implications for detecting community changes. *Conservation Ecology* 5, 7.

Wills, A.B., North, C., 1978. Problems of hybrid seed production. *Acta Horticulturae*. 83, 31–36.

Witter, S., Blochtein, B., 2003. Effect of pollination by bees and other insects on the production of onion seeds. *Pesquisa Agropecuária Brasileira* 38, 1399–1407.

World Wide Fund for Nature, 2010. *Living Planet Report 2010*. Gland.

Woyke, H.W., 1981. Some aspects of the role of the honeybee in onion seed production in Poland. *Acta Horticulturae*. 111, 91–98.

2

ANTHOPHILOUS DIVERSITY AND ABUNDANCE WITHIN ONION HYBRID SEED CROPS IN SOUTH AFRICA: GRADIENTS OF PERCENTAGE NATURAL HABITAT AND DIFFERING FARM MANAGEMENT PRACTICES

ABSTRACT

Pollination is a regulating ecosystem service supported by diverse natural habitats, and delivered to blooming agricultural crops by direct and indirect routes. Flower-visiting insect assemblages, especially bees and including wild honeybees, deliver direct pollination services to agricultural crops. Natural habitats can be used to support managed honeybee colonies by offering floral resources outside agricultural blooming periods and thereby deliver indirect pollination ecosystem services. The proximity of natural habitat to blooming crops has been suggested as a significant factor in determining the diversity of flower-visiting communities within crops. Here, I tested this hypothesis within the South African onion hybrid seed production industry. I found that, generally, insect diversity within onion hybrid seed crops was not dependent on the percentage of available natural habitat, while crop management practices significantly altered anthophile assemblages. The anthophile assemblage was generally highly species diverse. Blooming agricultural crops can play major roles in supporting insect assemblages, as the results reported here indicate for onion hybrid seed crops, especially in cultivated areas where floral resources are scarce. Onion hybrid seed growers in South Africa may therefore receive pollination ecosystem services from wild pollinator assemblages without the availability of extensive natural habitat.

INTRODUCTION

Agriculture is the human activity that has the most widespread and intense impact on natural landscapes with influences on local, regional and global scales (Matson *et al.*, 1997; Tilman *et al.*, 2001; Tscharntke *et al.*, 2005). Globally, the total area of land under cultivation from 1900 to 1990 has increased by 56%, while the global per capita cropland area decreased by half (Ramankutty *et al.*, 2002). On a local scale, agricultural intensification negatively impacts pollinator communities through habitat destruction and fragmentation and the use of agrochemicals (Brittain *et al.*, 2010; Kearns *et al.*, 1998; Kosior *et al.*, 2007; Potts *et al.*, 2010;

Richards, 2001; de Ruijter, 2002). Klein *et al.* (2007) reviewed sixteen studies on the effect of agricultural intensification on crop pollination at local and landscape scales. All the studies showed that agricultural intensification and degradation of habitat quality had a negative impact on the pollination of crops. In contrast, low-intensity land-use systems and organic farming appear to contribute positively towards pollinator conservation efforts with resultant improved pollination of crops (Tscharntke *et al.*, 2005; Gabriel & Tscharntke, 2007; Potts *et al.*, 2010; Jonason *et al.*, 2011). Several authors have linked pollinator diversity in crop fields and good quality adjacent source habitats to significant increases in crop yields (Carvalheiro *et al.*, 2010; Greenleaf & Kremen, 2006; Klein *et al.*, 2003; Kremen *et al.*, 2002; Olschewski *et al.*, 2006; Richards, 2001; Ricketts *et al.*, 2004; Steffan-dewenter & Tscharntke, 1999).

Insect diversity within onion hybrid seed crops

Hybrid onion (*Allium cepa* L.) seed production is entirely dependent on high insect pollinator activity to ensure cross pollination, seed set and profitable seed yields (Chandel, *et al.*, 2004; McGregor, 1976; Munawar *et al.*, 2011). The nectaries of *Allium* flowers are shallow and easily accessible to many types of pollinating insects, giving rise to a generalized entomophilous pollination syndrome (Brewster, 2008). A diversity of insect visitors to onion seed crops can thus be expected and have indeed been identified by several authors in various parts of the world (Bohart *et al.*, 1970; Caron *et al.*, 1975; Howlett *et al.*, 2005; Lederhouse *et al.*, 1968; Saeed *et al.*, 2008; Sajjad *et al.*, 2008; Williams & Free, 1974; Witter & Blochtein, 2003). Diptera and Hymenoptera species are often the most frequent visitors recorded on onion umbels (Free, 1993; Williams & Free, 1974; Witter & Blochtein, 2003; Howlett *et al.*, 2005; Saeed *et al.*, 2008; Sajjad *et al.*, 2008).

Honeybees as important pollinators of onion hybrid seed crops

Honeybees (*Apis mellifera* L.) are considered the most frequent and important visitors to onion crops with onion hybrid seed production across various parts of the world greatly dependent on pollination by honeybees. This is despite the fact that honeybees generally dislike onion nectar (Hagler *et al.*, 1990; Waller *et al.*, 1972), (an issue that will be discussed further in chapter 4). Areas of intensive crop cultivation are especially reliant on honeybees with onion hybrid seed crops usually stocking about 10 honeybee hives per hectare (Free, 1993; Lederhouse *et al.*, 1968; McGregor, 1976; Williams & Free, 1974; Woyke, 1981).

The honeybee contributes to crop pollination via two main routes. The first is through managed pollination services, where honeybee colonies are housed in Langstroth hives and supplied by commercial beekeepers to farmers in sufficient and readily available quantities to ensure pollination of their insect pollination dependent crops. Secondly, especially in regions where honeybees are indigenous, which includes Africa, Europe, and Asia (Hepburn & Radloff, 1998), honeybees form part of a pollination ecosystem service delivered by a suite of wild pollinator species to blooming crops. In South Africa, the Cape honeybee (*Apis mellifera capensis* Esch., Hymenoptera: Apidae) is indigenous and essentially endemic to the Western Cape Province (Hepburn & Radloff, 1998) and forms an important part of the wild pollinator community in the Western Cape, the region where onion hybrid seed are predominantly produced. It is also the species used by commercial beekeepers for managed crop pollination and honey production in the region. Therefore, when honeybees are collected in crops in the Western Cape, it is impossible to tell if they are from managed bee hives or from wild hives in the surrounding habitat. This means the pollination ecosystem service provided by wild honeybees cannot be quantified when managed honeybees are rented.

Aims

The aim of this chapter is to assess the diversity of anthophile insects that potentially could visit the flowering umbels of onion hybrid seed crops and provide an ecosystem service, and attempt to identify the factors that explain this diversity. My main hypothesis is that higher percentages of undisturbed, natural habitat within close vicinity of a crop should result in a greater diversity of anthophile insects within the crop, as natural vegetation should act as source habitat for anthophile insects, and therefore greater potential to provide an ecosystem service. I test this by sampling anthophile assemblages across gradients of percentage natural habitat next to crop fields and I describe and compare honeybee abundance to non-*Apis* anthophile abundances to determine the relative importance of non-*Apis* flower visitors as potential pollinators, in the context of gradients of percentage natural vegetation.

METHODS

Study Area

The main areas of onion hybrid seed farming are the Klein Karoo and southern Karoo regions. The Klein Karoo is an oblong region of about 23 500 km^2 (Vlok and Schutte-Vlok, 2010), representing an extended valley that stretches from east to west between two

mountain ranges parallel to the South African south coast, the Langeberg-Outeniqua mountain range in the south and the Witteberg-Swartberg mountain range in the north. The mountain slopes are characterized by nutrient-poor sandstone soils hosting fynbos vegetation while the low-lying areas generally have nutrient-rich loamy to clayey soils hosting succulent karoo vegetation types (Vlok & Schutte-Vlok, 2010). Three biomes meet in the Klein Karoo; namely the Fynbos, Succulent Karoo and Subtropical Thicket biomes (Mucina & Rutherford, 2006). The Fynbos and Succulent Karoo biomes are recognised as global biodiversity hotspots (Myers *et al*., 2000). The Succulent Karoo vegetation is characterized by open to sparse dwarf (up to 1 m tall) shrublands dominated by stem and leaf succulents and some fine-leaved evergreen shrubs (Hilton-Tailor & Le Roux, 1989).

The Klein Karoo has a semi-arid climate which is dominated by orographic rainfall gradients and rain shadow effects, with the mountain ranges receiving above 1 000 mm of rain annually, while the low-lying central valley receives 100-300 mm per year (Le Maitre *et al*., 2009). The rainfall season varies from west to east throughout the Klein Karoo, whereby the western region (Montagu) receives winter rainfall which progressively shifts to summer rainfall events received by the east (Willowmore) (Le Maitre *et al*., 2009). The daily temperature range between mean maximum temperatures above 30 °C in February to about 20-22 °C in August (Le Maitre *et al*., 2009).

The southern Karoo is a semi-arid region stretching northward from the Witteberg-Swartberg mountain range (CEPF, 2003) and is dominated by Succulent Karoo vegetation (Tainton, 1999). The Karoo has shallow, weakly developed alkaline soils (Cowling, 1986) and is generally not suitable for cultivation, except for alluvial terraces which have been utilized extensively (Dean & Milton, 1995). As with most arid and semi-arid regions, the southern Karoo is characterized by extreme temperatures and unpredictable and highly variable rainfall receiving approximately 170 mm annually (Cowling, 1986; Dean & Milton, 1995). Detailed descriptions of the ecology, geography and climatology of the Klein Karoo and southern Karoo are given by Cowling (1986); Cowling *et al*. (1986, 1997); Cowling & Roux (1987); Dean & Milton (1999), and Vlok & Schutte-Vlok (2010). These areas suit the climatic requirements for onion seed production which include low humidity and mild cool temperatures during the initial growth phase, followed by increased temperatures later on to induce flowering and support pollinator activity (Shanmugasundaram, 1998).

Selection of Farm Sites

Several of the main, commercial seed companies operating in the Karoo region were approached during the study to collaborate in strategic planning and to identify suitable onion hybrid seed farms for data collection. Eighteen farms growing onion hybrid seed, and located in the Klein Karoo and southern Karoo regions were identified and used for data collection during the flowering periods (late October through November) of 2009 and 2010 (refer to Figure A.1 & Table A.1 in Appendix A). It proved to be very difficult to nearly impossible to obtain true or definite cultivar information from the participating seed companies and seed growers due to confidentiality of varietal crosses used to create hybrids and competition between seed companies. Therefore, distinguishing varietal differences between the farms was based solely on coded information given by seed companies i.e. farm sites were able to be classed based on which variety was grown, but no other information on the variety or crosses used were given.

Natural habitat surrounding farm sites

Each onion hybrid seed crop field that was used for data collection was demarcated spatially with a GPS (GPSmap 76, Garmin). The surrounding land cover within a radius of 500 m from the site edges was classified as natural or cultivated using ArcGIS 9.2 (ESRI, 2006). The National Land Cover layer with 1 minute resolution (bgis.sanbi.org/landcover/project.asp) was used to classify the land cover surrounding the sites. From this, the percentage natural habitat surrounding each site within a 500 m radius was calculated and used as a predictor variable in data analyses.

Managed hive stocking densities

Numbers of managed hives placed within a 1 km radius from the experimental field sites were counted. Ribbands (1951) reported that hived honeybee colonies had substantial losses in weight at foraging distances further than 1 km. Furthermore, Gary *et al.*, (1972) reported that the average foraging range of honeybees from distant apiaries to onion crops was 557 m because of the relative unattractiveness of onion nectar to the bees. Hives were regarded as managed if honey was harvested from it, i.e. hives were maintained and serviced. Managed hive stocking densities were then calculated for each site by dividing the number of hives by the total hectares of blooming onion hybrid seed crop at each experimental site. No other cultivated crops bloomed at the same time as the hybrid onions.

Climatic variables on days of sampling

Data were collected during peak flowering (50% florets open) periods and on sunny days with optimal weather conditions for anthophile activity (temperatures above 20˚C and wind speeds below 10 km/hour). Ambient temperature (˚C) and wind speed (km/hour) were recorded throughout each sampling day with a hand held weather tracker (Kestrel 4000, Nielsen-Kellerman, U.S.A.). Mean daily temperature and wind speed were then calculated for each sampling day.

Farm management practices

Methods of irrigation used on the onion crops were noted and classified as drip (D), sprinkle (S) or flood (F) irrigation. The health of some crops could be described as sub-standard because of insufficient weed control and the possible presence of disease, and subsequently some of the crops deteriorated to sub-standard health levels at the time of seed harvest. Therefore, the health of each site was classified as good (G) or sub-standard (SS).

Sampling and collection of anthophile assemblages

Coloured pan traps with a diameter of 16 cm and a depth of 4 cm were used to sample flower-visiting insects within onion hybrid seed crops. Edge effects were avoided by keeping a buffer zone of at least 10 m from the edge of each experimental field. Five groups with six different coloured traps per group (red, orange, pink, white, fluorescent yellow and blue) were positioned randomly in each field, and were placed on podiums to raise them to just below flower level (about 1 m). Coloured pan trapping is an efficient and cost-effective method for sampling flower-visiting insects, while Vrdoljak & Samways (2012) suggested using a combination of colours to catch rarer species that are excluded by using reflective colours alone. Traps within a group were spaced about 1.5 m apart. The traps were set up before 9h00, three quarters filled with weakly diluted soapy water (Kearns & Inouye, 1993), and left out for two days in 2009 and one day in 2010. Trapped insects were placed in 70% ethanol for later assessment and identification. All insects caught in the six pan traps within a single group of pan traps were pooled and treated as a single sample. It has been found that small arthropods (body widths <3 mm) played no role in onion pollination (Jones, 1923; Walker *et al.*, 2011). Therefore, all insect specimens with body lengths >5 mm were identified to morpho-species (a list of the identified taxa appears in Appendix B). Specimens with no obvious pollinator value, like spiders and caterpillars were discarded. However, insects that were found to be numerous on the umbels were regarded as having possible

pollination value (e.g. Hemiptera and members of Coleoptera), and these specimens were therefore retained as viable anthophile species. Reference collections of morpho-species were sent to various expert taxonomists for identification and were deposited in museum collections (see Appendix B).

Data Analysis

Sampling effort and calculation of richness estimates

Because of the difference in sampling effort between the two years in terms of the number of days the pan traps had been set out, the pan trap data were kept separate where analytical procedures could not be used to standardize the samples across years. Rarefaction methods allow for meaningful standardization and comparison of datasets (Gotelli & Colwell, 2001). Therefore, a single sample-based rarefaction curve was calculated, based on the complete pan trap data matrix, using EstimateS™ v.8.2.0 software (Colwell, 2006), to evaluate the strength of the sampling effort in capturing the true species richness present in onion hybrid seed crops. The samples were randomized 50 times without replacement. Estimated species richness was also calculated using the EstimateS™ v.8.2.0 software (Colwell, 2006) and the results of the non-parametric incidence-based estimators ICE, Chao2 and Jacknife2 are reported. Chao2 were computed using the classic formula instead of the bias-corrected formula (Colwell, 2006). Incidence-based richness estimators have been reported to have higher precision in cases of low sampling intensities (Hortal *et al.*, 2006), which can also be accepted for the pan trap sampling which focussed solely on visitors within onion hybrid seed crops within a broader and probably more diverse mosaic landscape and only covered a limited period of sampling time. Incidence species richness estimators are also less sensitive to sample coverage, and distribution patchiness of species (see Hortal *et al.*, 2006).

Anthophile abundances and species diversity

Data for 2009 and 2010 were analysed separately. Non-parametric Wilcoxon Mann-Whitney rank-sum tests (linked with Monte-Carlo re-sampling to compute the null distribution of the test statistic (Hothorn *et al.*, 2008)) were used to test for significant differences in abundances for different insect guilds in the pan traps. The analyses were stratified by samples from the same sites. The "wilcox_test" function from the "coin" package (Hothorn *et al.*, 2012) was used in R (R Development Core Team, 2011). Insect order

abundance and species richness data were transformed to normality where necessary to do simple linear regressions or generalized linear regressions where appropriate, to test the relationships between abundance and species richness with percent natural habitat. Simple linear regression was applied to investigate the relationship between the over-all species diversity (H') measured at each site and percentage natural habitat.

Similarities and dissimilarities in anthophile assemblages across gradients of natural habitat and farm management practices

To examine the degree of similarity of the anthophile assemblages between the sites a hierarchical cluster analyses with group-average linkage was performed for the 2009 and 2010 pan trap datasets separately, using PRIMER v6.1.14 of PRIMER-E Ltd. (Clarke & Gorley, 2006). The species abundance matrices were first square root transformed to down weight the more abundant species. Resemblance matrices (between samples) were constructed based on Bray-Curtis similarities (Bray & Curtis, 1957). To find significant differences between samples, similarity profile permutation tests (SIMPROF) were linked with the cluster analyses and were set to perform 1000 permutations and 999 simulations with a significance level of 5%.

To enable the observation of main patterns and structures in the multivariate data cloud, principal coordinates analyses (PCO) (Gower, 1966) were used to construct unconstrained ordinations of the two pan trap datasets, based on Bray-Curtis resemblance matrices, using the PERMANOVA+ v1.0.4 add-on package to PRIMER v6.1.14 (Anderson *et al.*, 2008). PCO places the samples onto euclidean axes using a matrix of inter-point dissimilarities (Anderson *et al.*, 2008).

Distance-based linear models were constructed using the DistLM routine from the PERMANOVA+ v1.0.4 add-on package in PRIMER v.6.1.14 (PRIMER-E Ltd.) to identify important predictor variables that explained the anthophile assemblages. The predictor variables included percent natural habitat, managed hive stocking density, mean daily temperature, mean daily wind speed, field size, method of irrigation and field health. Draftsman plots and Pearson correlations were used to examine the continuous explanatory variables for collinearity and approximate normality. None of the variables were highly correlated ($r > 0.9$) and all were fairly symmetrically distributed across the range of each variable, thus no transformations were necessary. The continuous predictor variables were normalized by subtracting the mean from each entry for a single variable and further dividing

it by the standard deviation. This was done in order to place the variables on a common scale. Step-wise selection procedures were used to select the predictor variables in sequential tests together with the AIC (Akaike Information Criterion; (Akaike, 1974)) selection criterion to select the best models for both years.

The DistLM routine also implements distance-based redundancy analyses (dbRDA) which is used to construct a constrained ordination of the fitted values from the multivariate regression model in order to visualize the model in multivariate space (Anderson *et al.*, 2008). dbRDA is a nonparametric multivariate analysis using permutation tests to test for significance (McArdle & Anderson, 2001). dbRDA constrained ordinations are presented for both years.

Explaining the abundance of honeybees

Generalized linear mixed-effects models (GLMM), fitted by the Laplace approximation, with Poisson error distributions and log link functions were used to test the effects of the different explanatory variables on honeybee abundance in the pan trap samples taken in 2009 and 2010 respectively. The fixed variables tested were percent natural habitat, managed honeybee hive density, mean daily temperature, mean daily wind speed, method of irrigation and field health. Spearman rank correlations were used to test for collinearity between the continuous fixed variables with $r_s > 0.7$ used to indicate strong collinearity (Zuur *et al.*, 2009). GLMM models correct for over-dispersion by allowing the use of a random effect in the model (Zuur *et al.*, 2009). The sampling sites were used as random effects in both models. R v.2.14.1 statistical software was used (R Development Core Team, 2011) with the "lmer" function from the "lme4" package (Bates *et al.*, 2011). Backward elimination selection procedures were followed for model simplification, starting with a model including all explanatory variables as fixed terms and dropping the least significant term or the term which resulted in a lower AIC value, after testing the reduced model to the full model using ANOVA (Zuur *et al.*, 2009). If dropping a term did not result in a significant change ($P < 0.05$) in the model, the reduced model was retained.

RESULTS

Sampling effort and calculation of richness estimates

The sample-based rarefaction curve of all the pooled pan trap data did not reach a clear asymptote, and as expected, indicates that the sampling effort was not sufficient to capture the total diversity of flower-visiting insects that can be associated with onion hybrid seed crops (Figure 2.1). 44.4% (140 of 315) of the species caught were singletons which more or less correlates with the number of singletons usually present within diverse tropical forests (Novotny and Basset, 2000). Moreover, it should be noted that the sampling effort did not aim at capturing the diversity of the entire region involved, but only the diversity of anthophiles visiting onion hybrid seed crops. Nevertheless, the high percentage of singletons does indicate that the region's diversity of flower-visiting insects is probably richer than what is represented in the onion hybrid seed crops.

The estimated species richness (ICE = 519.98; Chao2 = 544.14 $\pm$ 53.86; & Jacknife2 = 455.42) calculated for the pooled pan trap data was considerably higher than the observed species richness (S_{obs} = 315), which also indicates that the actual diversity of anthophiles was probably not completely represented by the sampling effort. This could be ascribed to the large number of rare species (Singletons = 140, 40% and above) observed in the data set, for the richness estimators make use of these values to estimate real species diversity (Colwell and Coddington, 1994).

Anthophile abundance and species diversity

2009

Diptera was the most abundant insect order caught in the pan traps with 1,819 individuals sampled (Table 2.1, Figure 2.2). Hymenoptera was the second most abundant order with 1,695 individuals, of which most were honeybees (1,420 individuals). Diptera abundance and species richness were considerably higher at the two sites with the lowest percent natural habitat (1 and 2% respectively), with considerably fewer individuals and species present at the other sites (Table 2.1). Honeybee abundance seemed to be more constant across all sites (Table 2.1). Diptera was also the most species rich group overall, followed by non-*Apis* Hymenoptera (Table 2.1, Figure 2.2). Twenty nine bee species (58% of Hymenoptera) were present of which 12 species (41%) were represented by only one or two individuals, and therefore can be considered as rare species. Coleoptera, Hemiptera and Lepidoptera species

had low abundances and species richness (101, 105 and 78 individuals, respectively), with no significant differences in their overall abundances (Coleoptera vs. Hemiptera: Z = -0.74, P = 0.47; Coleoptera vs. Lepidoptera: Z = 1.30, P = 0.20; Hemiptera vs. Lepidoptera: Z = 1.95, P = 0.06) (Table 2.1).

Of the Diptera, Muscidae and Calliphoridae were the two most abundant families (50% and 18%, respectively). Hymenoptera was dominated by Halictidae species (73%), while Chalcididae accounted for 9% of total Hymenoptera abundance. Coleoptera was mostly represented by Coccinellidae (53%) and Chrysomelidae (25%). Only one butterfly species was caught while the rest of the Lepidoptera were moths.

Anthophile diversity had a weak negative relationship with percent natural habitat which was not significant (H'($\log_e$) = 2.94 – 0.01 x percent natural habitat, $F_{1,5} = 5.55$, P = 0.07, r^2 = 0.53). In most cases, the abundance and species richness of the insect orders decreased with percent natural habitat, except for Hymenoptera abundance which increased with percent natural habitat but not significantly so (for statistics see Table 2.2).

2010

Hymenoptera was the most abundant insect order caught in the pan traps with 1,454 individuals sampled, of which most were honeybees (978 individuals). Diptera and Coleoptera were also abundant with 678 and 558 individuals, respectively (Table 2.1, Figure 2.3). Honeybee abundance was significantly different from all insect order abundances, except Diptera (Z = -1.73, P = 0.09). There were no significant differences in the overall abundances of Diptera, non-*Apis* Hymenoptera and Coleoptera (Diptera vs. non-*Apis* Hymenoptera: Z = 1.71, P = 0.08; Diptera vs. Coleoptera: Z = -0.86, P = 0.40; non-*Apis* Hymenoptera vs. Coleoptera: Z = 0.78, P = 0.46), and also none between Hemiptera and Lepidoptera (Z = -0.01, P = 1) which had very low to no representation in the samples (less than 10 individuals each). Hymenoptera was the most species rich group, followed by Diptera and Coleoptera (Table 2.1, Figure 2.3). Of Hymenoptera, forty five species (58%) were bees of which 18 (40%) species were represented by one or two individuals and can therefore be considered as being rare.

Non-*Apis* Hymenoptera abundance was dominated by Halictidae (74%), followed by Colletidae (6%). Bombyliidae was the most abundant Diptera with 65% of the individuals, followed by Muscidae with 13% of the individuals. For Coleoptera, the most abundant

families were Coccinellidae and Buprestidae (51% and 22% of Coleoptera individuals, respectively).

Anthophile diversity had a weakly negative relationship with percent natural habitat which was not significant ($H'(\log_e) = 2.50 - 0.003$ x percent natural habitat, $F_{1,9} = 0.39$, $P = 0.54$, $r^2 = 0.04$). Again, the abundance and species richness of almost all insect orders decreased with increased percentage natural habitat, with Diptera abundance being an exception to the general trend (for statistics see Table 2.2).

Similarities and dissimilarities in anthophile assemblages across gradients of natural habitat and farm management practices

2009

A dendogram of the 2009 pan trap data (Figure 2.4), groups all the samples from each site together, while the SIMPROF test found the samples from the same sites to be distinct from the rest, except for sites C and F which were 46.5% similar with no significant difference between them ($\pi = 1.22$, $P = 11.9$). Samples from sites A and B, the two sites with the lowest percentage natural habitat, was 52.6% similar, the highest similarity between any of the sites, although they were significantly different from one another ($\pi = 2.05$, $P = 0.1$). In addition, samples from sites A and B were the least similar to the samples of the rest of the sites at 30% ($\pi = 3.69$, $P = 0.1$).

An unconstrained principal coordinates analysis (PCO) ordination grouped the sites into three distinct groups (Figure 2.5). The first two axes of the PCO explained 40.2% of the total variability in the multivariate data cloud (Figure 2.5). Multiple partial correlations of the explanatory variables with the two PCO axes indicated that percentage natural habitat (NatHab) and field health (Health-G/-SS) had the highest correlations with PCO axis 1 ($r^2 = 0.60$ and 0.41 respectively). Method of irrigation (Water-D/-S) had the highest correlation with PCO axis 2 ($r^2 = 0.54$), but also correlated with PCO axis 1 ($r^2 = 0.26$). Therefore, along the first PCO axis, the samples are split based on percentage natural habitat and field health, with good health and high percentage natural habitat to the right. Sites A, B and D are split from sites C, E, F and G based on irrigation methods used (sprinkle and drip irrigation, respectively).

The best model selected all seven explanatory variables and explained 61.3% of the total variation in the multivariate data cloud (AIC = 245.66, df = 7). Field health has been found to

be the most important variable, explaining 25.6% of the variability in species diversity, followed by method of irrigation that explained 10.7%. Together, these two variables alone explained 36.3% of the variation. Table 2.3 lists the explanatory variables in the order they have been added to the model to improve the selection criterion. The first two axes of the dbRDA constrained ordination explained 63.3% of the fitted variation in the model, and 38.8% of the total variation in the multivariate data cloud (Figure 2.6). The ordination shows similar patterns as those projected by the PCO ordination, which means that it can be accepted that the model explains most of the salient variation in the multivariate data cloud (Anderson *et al.*, 2008). A vector overlay of the multiple partial correlations of $r > 0.2$ of individual species are also shown in Figure 2.6 and the values are listed in Table 2.4. Muscid and calliphorid flies were highly represented in samples taken at sites with sub-standard health and low percentage natural habitat, while *Halictus* bees were mostly present at sites with drip irrigation (Figure 2.6, Table 2.4).

2010

The cluster analysis of the 2010 data grouped the samples into four distinct groups (a – d) (Figure 2.7). No significant differences were found between the samples in group **b** (sites L – Q,, $\pi = 0.91$, $P = 16.6$) and between the samples in group **d** (sites I – K, $\pi = 0.79$, $P = 50.5$). The samples in group **b** are from sites with high percentages of natural habitat while samples in group **d** are from three sites with intermediate percentages of natural habitat. Group **a** represents the samples taken at the site with the highest percentage natural habitat (91%) and it was the least similar to each other (25.6%) and significantly different from all the other sites ($\pi = 2.29$, $P = 0.1$). Group **c** includes the samples from the site with the lowest percentage natural habitat (3%) which was significantly different from the other groups ($\pi = 1.58$, $P = 1.1$).

A PCO ordination grouped the samples roughly into three groups, with no distances between the cluster groups **c** and **d**, and a continuum of samples from the cluster groups **a** and **b** (Figure 2.8). The first two PCO axes explained only 33.8% of the total variability in the multivariate data cloud. Multiple partial correlations of the explanatory variables with the two PCO axes indicate that percentage natural habitat (NatHab) correlated strongly with the first PCO axis ($r^2 = 0.71$). The strongest correlations with PCO axis 2 were drip-irrigation ($r^2 = -0.44$) and wind speed ($r^2 = -0.42$). Therefore, cluster groups **a** and **b** were separated from groups **c** and **d** along PCO axis 1, from sites with high percent natural habitat to the right, to

sites with lower percentage natural habitat to the left (Figure 2.8). Wind and method of irrigation appeared to be the factors splitting samples along the second PCO axis.

The best model selected all seven explanatory variables and explained 46.4% of the total variation in the multivariate data cloud (AIC = 406.85, df = 9). Percentage natural habitat and method of irrigation were the most important variables, each explaining 12.1% of the species diversity patterns. The order of selection of the remaining explanatory variables is given in Table 2.3. The first two axes of the dbRDA constrained ordination explained 61.6% of the fitted variation in the model, and 38.8% of the total variation in the multivariate data cloud (Figure 2.9). A vector overlay of the multiple partial correlations of $r > 0.2$ of individual species are shown in Figure 2.9 and the values are listed in Table 2.4. *A.m. capensis* (Hymenoptera), *Neomyia peronii* Robineau-Desvoidy (Diptera: Muscidae), *Lasioglossum* sp.14 & 15 (Hymenoptera: Halictidae), *Pteraulax* sp. (Diptera: Bombyliidae) and Buprestidae sp.1 (Coleoptera) seemed to characterize samples taken at sites with low percentage natural habitat, splitting sites along the first dbRDA axis. The rest of the species characterized sites with high percentage natural habitat and different irrigation methods (see Table 2.4).

Explaining the abundance of honeybees

None of the fixed continuous variables were highly correlated and therefore all fixed variables were included in the 2009 and 2010 models. No significant relationships were found between 2009 honeybee abundance and managed hive stocking densities or percentage natural habitat (Table 2.5). Honeybee abundance in 2009 seemed to respond more readily to daily weather conditions, with abundances decreasing significantly with increases in both ambient temperature and wind speed. Honeybee abundance was higher in fields with sprinkle irrigation than in fields with drip irrigation, but not significantly so.

In 2010, honeybee abundance responded significantly negatively to higher managed hive stocking densities (Table 2.5). Although negative relationships were found between honeybee abundance and percentage natural habitat, ambient temperature and field size, none of them were significant. Fields with sprinkle irrigation had greater honeybee abundance, while fields with flood irrigation had the lowest honeybee abundance, although none of these relationships were significant (Table 2.5).

DISCUSSION

Anthophile abundance and species diversity

Based on the pan trap results, a highly diverse anthophile community was present within the onion hybrid seed crops during flowering (2009: H' = 3.89; 2010: H' = 3.57), with honeybees being the most abundant species encountered in both sampling years. This is in accordance with other findings for hybrid onions (Bohart *et al.*, 1970; Caron *et al.*, 1975; Chandel *et al.*, 2004; Saeed *et al.*, 2008; Sajjad *et al.*, 2008). The anthophile community was mostly represented by species of Hymenoptera, Diptera and Coleoptera, while Hemiptera and Lepidoptera were lower in number. Vrdoljak & Samways (2012) reported that species of butterflies were strongly under-represented in pan trap samples taken in the Cape Floristic Region (using a selection of colours similar to the colours used here) and did not represent the actual butterfly population. Therefore, pan trap catches generally under-represent the true butterfly community. However, these results corresponds to those found by Lederhouse *et al.* (1968) who reported that, of the 1,172 insects collected from hybrid onion umbels, 68 were Hemipteran species (5.8%), and 9 were Lepidopteran species (0.77%).

In both years, the proportional contribution of honeybees, and the most abundant families representing Hymenoptera and Coleoptera, were very similar. Honeybees accounted for about 35% of total anthophile abundance, while Halictid bees accounted for 70% of non-*Apis* bee abundance in both years. Also, ladybird beetles accounted for 50% of Coleoptera abundance in both years. However, for Diptera, the most abundant families differed for each year. Muscidae was the most abundant family in 2009, while Bombyliidae was the most abundant family in 2010.

In general, anthophile diversity was not significantly negatively affected by the extent and proximity of the agricultural matrix surrounding field sites and correspondingly, appeared not to be influenced by the percentage of undisturbed natural habitat surrounding field sites. These findings are in contrast to those that have been generally found to show increases in insect pollinator abundance and richness within crop fields that are in close proximity to natural habitat, and decreases in insect pollinator abundance and richness for crop fields that are increasingly isolated from natural vegetation (Heard and Exley, 1994; De Marco and Coelho, 2004; Ricketts, 2004; Blanche and Cunningham, 2005; Blanche *et al.*, 2006; Chacoff and Aizen, 2006; Carvalheiro *et al.*, 2010; Klein *et al.*, 2012).

For non-*Apis* Hymenoptera, and especially bees, the similarly high numbers of species and abundances found across gradients of percentage natural vegetation suggests that at least some non-*Apis* bees are able to survive within the agricultural matrix associated with onion hybrid seed crops, finding adequate floral resources and nesting sites. Similar findings have been made for sunflowers, where ground-nesting bee species were observed nesting and surviving in cultivated areas where remnants of natural habitat prevailed (Kim *et al.*, 2006). It was noted that within South African onion hybrid seed crops, even those study sites that were situated within highly cultivated areas had some small fragments of mostly undisturbed habitat close by; thus, possibly offering some habitat resources to bee species living within an agricultural matrix. A study that compared wild bee diversity over a period of 15 years showed that the persistence of bee fauna was largely supported by the mosaic structure of the landscape which included remnants of natural and semi-natural plant communities which provided refuge habitat in the midst of intensive agricultural management (Banaszak, 1992). Many of the crops used in this study were situated in alluvial terraces along water courses, like most other cultivation activities in the semi-arid succulent Karoo (Dean and Milton, 1995; Thompson *et al.*, 2005). This is also where several bee and aculeate wasp species make their nests because of the availability of water (Gess, 2001).

Coleoptera, Hemiptera and Lepidoptera were more diverse in cultivated areas across both years of sampling. The three insect orders were, however, mainly represented by species that could be regarded as pest species (e.g. moths) or those that prey on pest species (e.g. ladybird beetles), and whose numbers might have been directly or indirectly ameliorated at sites with high percentage natural habitat, hosting natural parasitoids and predator species (Pimentel *et al.*, 1992, 1997; Tilman *et al.*, 2002; Olson & Wäckers, 2007). Booij and Noorlander (1992) showed that ground-dwelling polyphagous predators (e.g. ground beetles and rove beetles) were more abundant in organic and irrigated onion crops than in conventional onion crops.

Overall, the structure and density of the anthophile communities within onion hybrid seed crops varied over time and space, supporting the findings of Benedek (1976), who found that the abundance and species richness of onion pollinating insects varies greatly both spatially and temporally. He attributed this fact to the lack of specialized pollinators associated with onion flowers. This is a strong disadvantage for crop pollination, as farmers may not know if sufficient wild pollinators would be available in a given place and time. In this study, the sampling sites were spread over a wide geographical area (Figure A.1 in Appendix A). Gess (2001) and Colville *et al.* (2002) reported high beta-diversity and species turn-over of solitary

bees, aculeate wasps and monkey beetles in the semi-arid Succulent Karoo biome. High levels of anthophile beta-diversity, coupled with the fact that onion flowers have a generalized floral phenology may explain the variability of anthophile communities present within onion hybrid seed crops in the succulent Karoo.

Similarities and dissimilarities in anthophile assemblages across farm management practices

Apart from regional and temporal variation in species diversity, the two farm management practices that were quantified here appeared to have played an important role in determining anthophile community structure at the sites. Houseflies and blowflies characterized sites in cultivated areas in 2009 and this could be ascribed to the higher densities of ostriches in these areas at the time of sampling. Ostrich farming is one of the major farming practices in the Klein Karoo region (Beyleveld, 1967). These sites also happened to be sub-standard in health, possibly further encouraging the high abundance of flies breeding on diseased and rotting onion plants.

The methods used to irrigate crop fields, determine to a large extent the availability of water to anthophile insects (Gess, 2001) and may also change the availability of nectar resources through changes in onion nectar viscosity, especially under sprinkle irrigation (Brown *et al*., 1977; Mayer & Lunden, 2001). Sprinkle irrigation could also influence the availability of pollen as the pollen can become wet and sticky and therefore unattractive to certain flower visitors. Halictid bees were more abundant in crop fields with drip irrigation systems, suggesting that they were probably foraging for dry pollen on the male-fertile umbels. This is in accordance with findings made by Ngamo *et al*. (2007) who reported that halictid bees mainly foraged during higher temperatures and lower relative humidity to collect dry pollen from *Brachiaria ruziziensis* R. flowers (Poaceae). Even though sprinkle irrigation might deter some pollen foragers, hybrid onion crops are predominantly pollinated by insects foraging for nectar resources because of the male-sterility of the male-sterile umbels (McGregor, 1976). Two housefly species, two ladybird beetle species and one blister beetle (Meloidae) species characterized fields with sprinkle or flood irrigation, suggesting that they might have been attracted to the diluted nectar sources, or to the water source which were more readily available for consumption. Meloidae adults are known to feed on flowers, foliage and nectar (Picker *et al*., 2004), while the diet of predaceous Coccinellidae

beetles can also include non-prey food, such as nectar, pollen, foliage and fungi (Lundgren, 2009).

Explaining the abundance of honeybees

Honeybee abundance within the crops was primarily determined by climatic conditions in 2009, but not in 2010. All mean daily temperatures recorded on data sampling days were above 20 ˚C, while mean daily temperatures above 30 ˚C were recorded at some sites. Martins (2004) suggested that honeybees were susceptible to heat stress on hot days, and as a consequence, they became less active as temperatures rose above about 26 ˚C in an arid climate. Ngamo *et al*. (2007) also found that all insect visitors to *Brachiaria ruziziensis* (Poaceae) in Cameroon stopped foraging after 11h30 when temperatures became too hot, averaging at 34.4 ˚C. This might explain lower honeybee densities within the hybrid onion crops on days when higher mean temperatures were recorded. Pollinator visitation decreases as wind speed increases (Kearns & Inouye, 1993), and this was reflected in lower honeybee abundance when wind speeds were higher.

One would expect that honeybee densities would be greater at sites where more managed hives were deployed. However, this was the exact opposite in 2010. A possible explanation for the negative relationship between honeybee abundance and managed hive stocking density might be that hived bees did not prefer working on the onion inflorescences as a primary food source in 2010, and rather relied on stored food resources or sought floral resources elsewhere. It might also be that increased competition for hybrid onion flower resources as a result of higher managed hive stocking densities could have caused honeybees to seek floral resources elsewhere. Even though there was a negative relationship between managed hive density and honeybee abundance in the pan traps in 2009, it was not significant.

CONCLUSION

Despite high honeybee abundance, a diverse anthophile community was present within onion hybrid seed crops across varying gradients of percentage natural habitat, which suggests that there is potential for pollination ecosystem services to be gained by onion hybrid seed growers irrespective of the amount of natural vegetation close by. However, anthophile species and abundance varied both spatially and temporally between onion fields

sampled in the study region, with sites generally distinct from each other and farm management practices playing a significant but small role in determining the structure of anthophile communities. Farmers will thus not have any indication if sufficient flower visitors are available at any given time and place. It is also expected that different species and families would vary in their importance as actual pollinators because of life history differences. It is also likely that the type of farming practices listed is selected for other reasons that prevent farmers from changing these practices to benefit potential pollinators. Regardless of these concerns, whether or not this potential ecosystem service is actually realised, and whether species from this community are actively using onion flowers as a forage resource is explored in Chapter 3 and 4.

REFERENCES

Akaike, H., 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716–723.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. *PERMANOVA+ for PRIMER: Guide to software and statistical methods*. PRIMER-E, Plymouth, UK.

Banaszak, J., 1992. Strategy for conservation of wild bees in an agricultural landscape. *Agriculture, Ecosystems & Environment* 40, 179–192.

Bates, D., Maechler, M., Bolker, B., 2011. lme4: Linear mixed-effects models using S4 classes.

Benedek, P., 1977. Behaviour of honeybees (*Apis mellifera* L.) in relation to the pollination of onion (*Allium cepa* L.) inflorescences. *Zeitschrift fur Angewandte Entomologie* 82, 414–420.

Benedek, P., Gaal, E., 1972. The effect of insect pollination on seed onion, with observations on the behavior of honey bees on the crop. *Journal of Apicultural Research* 11, 175–180.

Beyleveld, A.J., 1967. Ostrich farming in South Africa. *Agrekon* 6, 14–16.

Blanche, K.R., Cunningham, S.A., 2005. Rainforest provides pollinating beetles for Atemoya crops. *Journal of Economic Entomology* 98, 1193–1201.

Blanche, K.R., Ludwig, J.A., Cunningham, S.A., 2006. Proximity to rainforest enhances pollination and fruit set in orchards. *Journal of Applied Ecology* 43, 1182–1187.

Bohart, G.E., Nye, W.P., Hawthorn, L.R., 1970. *Onion pollination as affected by different levels of pollinator activity*. Utah Agricultural Experiment Station Bulletin Nr. 482

Booij, C.J.H., Noorlander, J., 1992. Farming systems and insect predators. *Agriculture, Ecosystems and Environment* 40, 125–135.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs* 27, 325–349.

Brewster, J.L., 2008. *Onions and other vegetable Alliums, 2nd Edition*. CAB International, Oxon.

Brittain, C.A., Vighi, M., Bommarco, R., Settele, J., Potts, S.G., 2010. Impacts of a pesticide on pollinator species richness at different spatial scales. *Basic and Applied Ecology* 11, 106–115.

Brown, J., Wright, J.L., Kohl, R.A., 1977. Onion-seed yield and quality as affected by irrigation management. *Agronomy Journal* 69, 369–372.

Caron, D.M., Lederhouse, R.C., Morse, R.A., 1975. Insect pollinators of onion in New York State. *Hortscience* 10, 273–274.

Carvalheiro, L.G., Seymour, C.L., Veldtman, R., Nicolson, S.W., 2010. Pollination services decline with distance from natural habitat even in biodiversity-rich areas. *Journal of Applied Ecology* 47, 810–820.

CEPF, 2003. *Ecosystem profile: The Succulent Karoo hotspot, Namibia and South Africa, Africa*. Critical Ecosystem Parnership Fund.

Chacoff, N.P., Aizen, M.A., 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. *Journal of Applied Ecology* 43, 18–27.

Chandel, R.S., Thakur, R.K., Bhardwaj, N.R., Pathania, N., 2004. Onion seed crop pollination: a missing dimension in mountain horticulture. *Acta Horticulturae* 631, 79–86.

Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User manual/tutorial*. PRIMER-E, Plymouth.

Colville, J.F., Picker, M.D., Cowling, R.M., 2002. Species turnover of monkey beetles (Scarabaeidae: Hopliini) along environmental and disturbance gradients in the Namaqualand region of the succulent Karoo, South Africa. *Biodiversity and Conservation* 11, 243–264.

Colwell, R.K., 2006. EstimateS, version 8: Statistical estimation of species richness and shared species from samples.

Colwell, R.K., Coddington, J. A, 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical transactions of the Royal Society of London: Biological sciences* 345, 101–118.

Cowling, R.M., 1986. *A description of the Karoo Biome Project*. South African National Scientific Programmes Report No. 122. Pretoria.

Cowling, R.M., Richardson, D.M., Pierce, S.M. (Eds.), 1997. *Vegetation of southern Africa*. Cambridge University Press, Cambridge.

Cowling, R.M., Roux, P.W., 1987. *The karoo biome: a preliminary synthesis Part 2 - vegetation and history*. South African National Scientific Programmes Report No 142, Pretoria.

Cowling, R.M., Roux, P.W., Pieterse, A.I.H., 1986. *The karoo biome: a preliminary synthesis. Part 1 - physical environment*. South African National Scientific Programmes Report No 142, Pretoria.

De Marco, P., Coelho, F.M., 2004. Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity and Conservation* 13, 1245–1255.

De Ruijter, A., 2002. Pollinator diversity and sustainable agriculture in the Netherlands, in: Kevan, P., Imperatriz-Fonseca, V.L. (Eds.), *Pollinating bees: the conservation link between agriculture and nature*. Ministry of Environment, Brasilia, pp. 67–70.

Dean, W.R.J., Milton, S.J., 1995. Plant and invertebrate assemblages on old fields in the arid southern Karoo, South Africa. *African Journal of Ecology* 33, 1–13.

Dean, W.R.J., Milton, S.J., 1999. *The Karoo: ecological patterns and processes*. Cambridge University Press, Cambridge.

ESRI, 2006. ArcGIS, version 9.2. ESRI, Redlands, U.S.A.

Ewies, M.A., El-Sahhar, K.F., 1977. Observations on the behaviour of honeybees on onion and their effects on the seed yield. *Journal of Apicultural Research* 16, 194–196.

Free, J.B., 1993. Alliaceae, in: *Insect pollination of crops*. Academic Press, London, UK, pp. 115–121.

Gabriel, D., Tscharntke, T., 2007. Insect pollinated plants benefit from organic farming. *Agriculture, Ecosystems & Environment* 118, 43–48.

Gary, N.E., Witherell, P.C., Marston, J.M., 1972. Foraging range and distribution of honey bees used for carrot and onion pollination. *Environmental Entomology* 1, 71–78.

Gess, S.K., 2001. *The Karoo, its insect pollinators and the perils which they face*. International Pollinator Initiative (IPI) Case Study. (www.ecoport.org)

Gotelli, N.J., Colwell, R.K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4, 379–391.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53, 325–338.

Greenleaf, S.S., Kremen, C., 2006. Wild bee species increase tomato production and respond differently to surrounding land use in North California. *Biological Conservation* 133, 81–87.

Hagler, J.R., Cohen, A.C., Loper, G.M., 1990. Production and composition of onion nectar and honey bee (Hymenoptera: Apidae) foraging activity in Arizona. *Environmental Entomology* 19, 327–331.

Heard, T.A., Exley, E., 1994. Diversity, abundance and distribution of insect visitors to macadamia flowers. *Environmental Entomology* 23, 91–100.

Hepburn, H.R., Radloff, S.E., 1998. *Honeybees of Africa*. Springer Verlag, Berlin.

Hilton-Tailor, C., Le Roux, A., 1989. Conservation status of the fynbos and karoo biomes, in: Huntley, B.J. (Ed.), *Biotic diversity in southern Africa*. Oxford University Press, Cape Town, pp. 202–223.

Hortal, J., Borges, P. a. V., Gaspar, C., 2006. Evaluating the performance of species richness estimators: sensitivity to sample grain size. *Journal of Animal Ecology* 75, 274–287.

Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeileis, A., 2008. Implementing a class of permutation tests: the coin package. *Journal of Statistical Software* 28, 1–23.

Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeiteis, A., 2012. *Package "coin" - Conditional inference procedures in a permutation test framework*. R CRAN Repository

Howlett, B.G., Donovan, B.J., McCallum, J.A., Newstrom, L.E., Teulon, D.A.J., 2005. Between and within field variability of New Zealand indigenous flower visitors to onions. *New Zealand Plant Protection* 58, 213–218.

Jonason, D., Andersson, G.K.S., Ockinger, E., Rundlöf, M., Smith, H.G., Bengtsson, J., 2011. Assessing the effect of the time since transition to organic farming on plants and butterflies. *The Journal of Applied Ecology* 48, 543–550.

Jones, H.A., 1923. Pollination and self-fertility in the onion. *Proceedings of the American Society for Horticultural Science* 20, 191.

Jones, H.A., Emsweller, S.L., 1933. Methods of breeding onions. *Journal of Apiculture* 7, 625–642.

Kearns, C.A., Inouye, D.W., 1993. *Techniques for pollination biologists*. University Press of Colorado, Niwot.

Kearns, C.A., Inouye, D.W., Waser, N.M., 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology & Systematics* 29, 83–112.

Kim, J., Williams, N.M., Kremen, C., 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. *Journal of the Kansas Entomological Society* 79, 309–320.

Klein, A., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274, 303–313.

Klein, A.-M., Brittain, C., Hendrix, S.D., Thorp, R., Williams, N.M., Kremen, C., 2012. Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology* 49, 723–732.

Klein, A.-M., Steffan-Dewenter, I., Tscharntke, T., 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society B: Biological Sciences* 270, 955–961.

Kosior, A., Celary, W., Olejniczak, P., Fijal, J., Król, W., Solarz, W., Plonka, P., 2007. The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. *Oryx* 41, 79–88.

Kremen, C., Williams, N.M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences* 99, 16812–16816.

Le Maitre, D.C., Colvin, C., Maherry, A., 2009. Water resources in the Klein Karoo: the challenge of sustainable development in a water-scarce area. *South African Journal Of Science* 105, 39–48.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1968. Onion pollination in New York. *New York's Food and Life Sciences Bulletin* 1, 8–9.

Lundgren, J.G., 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. *Biological Control* 51, 294–305.

Martins, D.J., 2004. Foraging patterns of managed honeybees and wild bee species in an arid African environment: ecology, biodiversity and competition. *International Journal of Tropical Insect Science* 24, 105–115.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural Intensification and Ecosystem Properties. *Science* 277, 504–509.

Mayer, D.F., Lunden, J.D., 2001. Honey bee management and wild bees for pollination of hybrid onion seed. *Acta Horticulturae* 561, 275–278.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82, 290–297.

McGregor, S.E., 1976. Common vegetables for seed and fruit, in: McGregor, S.E. (Ed.), *Insect pollination of cultivated crop plants*. United States Department of Agriculture, Washington.

Mucina, L., Rutherford, M.C. (Eds.), 2006. *The vegetation of South Africa, Lesotho and Swaziland, Strelitzia 19*. South African National Biodiversity Institute, Pretoria.

Munawar, M.S., Raja, S., Niaz, S., Sarwar, G., 2011. Comparative performance of honeybees (*Apis mellifera* L.) and blow flies (*Phormia terronovae*) in onion (*Allium cepa* L.) seed setting. Journal of Agricultural Research 49, 49–56.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., De Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.

Ngamo, L.S.T., Tchuenguem, F.N.F., Mbofung, G., 2007. Diversity and feeding behaviour of insects visiting *Brachiaria ruziziensis* (Poaceae). *International Journal of Tropical Insect Science* 25, 214–217.

Novotny, V., Basset, Y., 2000. Rare species in communities of tropical insect herbivores: pondering the mystery of singletons. *Oikos* 89, 564–572.

Olschewski, R., Tscharntke, T., Benítez, P.C., Schwarze, S., Klein, A., 2006. Economic evaluation of pollination services comparing coffee landscapes in Ecuador and Indonesia. *Ecology And Society* 11, 7.

Olson, D.M., Wäckers, F.L., 2007. Management of field margins to maximize multiple ecological services. *Journal of Applied Ecology* 44, 13–21.

Picker, M.D., Griffiths, C., Weaving, A., 2004. *Field guide to insects of South Africa*. Struik Publishers, Cape Town.

Pimentel, D., Stachow, U., Takacs, D.A., Brubaker, H.W., Dumas, A.R., Meaney, J.J., O'Neil, J.A.S., Onsi, D.E., Corzilius, D.B., 1992. Conserving biological diversity in agricultural/forestry systems. *BioScience* 42, 354–362.

Pimentel, D., Wilson, C., McCullum, C., Huang, R., Dwen, P., Flack, J., Tran, Q., Saltman, T., Cliff, B., 1997. Economic and environmental benefits of biodiversity. *BioScience* 47, 747–757.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* 25, 345–353.

R Development Core Team, 2011. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. (www.r-project.org)

Ramankutty, N., Foley, J.A., Olejniczak, N.J., 2002. People on the land: changes in global population and croplands during the 20th century. *Ambio* 31, 251–257.

Ribbands, C.R., 1951. The flight range of the honey-bee. *Journal of Animal Ecology* 20, 220–226.

Richards, A.J., 2001. Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Annals of Botany* 88, 165–172.

Ricketts, T.H., 2004. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* 18, 1262–1271.

Ricketts, T.H., Daily, G.C., Ehrlich, P.R., Michener, C.D., 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences* 101, 12579–12582.

Saeed, S., Sajjad, A., Kwon, O., Kwon, Y.J., 2008. Fidelity of Hymenoptera and Diptera pollinators in onion (*Allium cepa* L.) pollination. *Entomological Research* 38, 276–280.

Sajjad, A., Saeed, S., Masood, A., 2008. Pollinator community of onion (*Allium cepa* L.) and its role in crop reproductive success. *Pakistan Journal of Zoology* 40, 451–456.

Shanmugasundaram, S., 1998. *Onion cultivation and seed production*. Shanhua, Tainan, AVRDC.

Steffan-Dewenter, I., Tscharntke, T., 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121, 432–440.

Tainton, N. (Ed.), 1999. *Veld management in South Africa*. University of Natal Press, Pietermaritzburg.

Thompson, M., Vlok, J., Cowling, R.M., Cundill, S.L., Mundau, N., 2005. *A land transformation map for the Little Karoo*. GeoterraImage, Pretoria.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418, 671–677.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven environmental change. *Science* 292, 281–284.

Tscharntke, T., Klein, A.-M., Kruess, A., Steffan-Dewenter, I., Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecology Letters* 8, 857–874.

Vlok, J., Schutte-Vlok, A.L., 2010. *Plants of the Klein Karoo*. Umdaus Press, Pretoria.

Voss, R.E., Murray, M., Bradford, K., Mayberry, K.S., Miller, I., 1999. *Onion seed production in California*. University of California, Devision of Agriculture and Natural Resources.

Vrdoljak, S.M., Samways, M.J., 2012. Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation* 16, 345–354.

Walker, M.K., Howlett, B.G., Wallace, A.R., Mccallum, J.A., Teulon, D.A.J., 2011. The diversity and abundance of small arthropods in onion, *Allium cepa*, seed crops, and their potential role in pollination. *Journal of Insect Science* 11:98, available online: insectscience.org/11.98

Waller, G.D., Carpenter, E.W., Ziehl, O.A., 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. *Journal of the American Society of Horticultural Science* 97, 535–539.

Williams, I.H., Free, J.B., 1974. The pollination of onion (*Allium cepa* L.) to produce hybrid seed. *Journal of Applied Ecology* 11, 409–417.

Witter, S., Blochtein, B., 2003. Effect of pollination by bees and other insects on the production of onion seeds. *Pesquisa Agropecuária Brasileira* 38, 1399–1407.

Woyke, H.W., 1981. Some aspects of the role of the honeybee in onion seed production in Poland. *Acta Horticulturae* 111, 91–98.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. *Mixed effects models and extensions in ecology with R*. Springer, New York.

Table 2.1: Abundances (mean ± S.D.) of honeybees and potential flower-visitors within each insect order, sampled in the field sites with pan traps. There are 5 samples per site and each sample consists of pooled abundances from six different-coloured pan traps. The total number of species sampled at each site are indicated in brackets. The percentage natural habitat surrounding each site within a 500m radius, and the Shannon diversity index (H', to the base $\log_e$) for each site are also indicated.

Year	Site	Natural habitat (%)	H'($\log_e$)	*A.m. capensis*	Diptera	non-*Apis* Hymenoptera	Coleoptera	Hemiptera	Lepidoptera
2009	B	1	3.37	33.80 ± 9.52	143.00 ± 12.71(59)	6.60 ± 3.21(15)	7.20 ± 3.77 (6)	5.80 ± 1.30 (8)	5.40 ± 3.36(10)
	A	2	2.93	52.80 ± 8.58	145.00 ± 25.96(40)	9.00 ± 5.10(13)	1.80 ± 1.30 (6)	1.20 ± 1.30 (3)	4.00 ± 2.24 (6)
	C	25	2.43	46.20 ± 3.49	26.00 ± 1.73 (29)	9.00 ± 5.79(11)	4.60 ± 1.67 (5)	2.60 ± 2.19 (3)	1.00 ± 0.71 (2)
	D	38	1.98	34.60 ± 9.79	11.80 ± 5.93 (21)	2.80 ± 0.84(11)	1.40 ± 1.14 (4)	4.80 ± 0.84 (6)	1.20 ± 0.84 (5)
	E	64	2.05	25.00 ± 6.04	6.00 ± 1.58 (13)	10.20 ± 3.83(16)	1.00 ± 1.00 (3)	1.20 ± 1.10 (1)	0.60 ± 1.34 (3)
	F	67	1.52	52.80 ± 7.07	14.78 ± 10.48(15)	6.20 ± 2.28(11)	1.40 ± 1.14 (3)	2.40 ± 1.67 (2)	1.20 ± 1.30 (5)
	G	85	2.44	38.80 ± 3.49	21.60 ± 8.91 (24)	11.60 ± 2.07(16)	2.80 ± 1.79 (6)	3.00 ± 2.55 (4)	2.20 ± 1.64 (5)
Overall abundance				*40.57 ± 6.85*†	*52.60 ± 9.61*†	*7.91 ± 3.30*†	*2.89 ± 1.69*‡	*3.00 ± 1.56*‡	*2.23 ± 1.63*‡
Total number of species				-	98	50	17	13	18
2010	H	3	2.86	7.80 ± 4.38	12.40 ± 3.51 (15)	14.60 ± 6.88 (9)	9.80 ± 2.77(10)	0.60 ± 0.89 (2)	1.40 ± 1.52 (3)
	I	39	2.55	16.40 ± 8.32	2.20 ± 1.79 (8)	8.20 ± 4.55(12)	12.00 ± 3.39(17)	0.00 ± 0.00 (0)	0.20 ± 0.45 (1)
	J	40	1.70	74.40 ± 22.80	5.00 ± 2.00 (15)	10.40 ± 5.37(15)	18.40 ± 5.32(19)	0.00 ± 0.00 (0)	0.20 ± 0.45 (1)
	K	72	2.13	16.40 ± 4.10	6.00 ± 3.08 (16)	4.60 ± 3.91(13)	5.40 ± 1.95 (5)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
	L	75	2.37	5.20 ± 5.50	15.29 ± 10.89(13)	15.80 ± 6.26(21)	15.60 ± 3.44 (8)	0.40 ± 0.55 (1)	0.00 ± 0.00 (0)
	M	77	1.86	13.00 ± 2.00	4.00 ± 1.41 (10)	2.40 ± 2.07(10)	7.40 ± 2.19 (4)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
	N	81	2.05	20.00 ± 6.04	23.60 ± 5.32 (18)	5.80 ± 1.92(18)	9.40 ± 5.94(15)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
	O	84	2.81	10.80 ± 4.38	2.40 ± 1.52 (10)	9.60 ± 4.45(17)	8.00 ± 2.83 (7)	0.20 ± 0.45 (1)	0.00 ± 0.00 (0)
	P	86	2.17	16.00 ± 1.87	15.60 ± 3.85 (10)	10.60 ± 2.61(15)	4.00 ± 2.00 (7)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
	Q	89	2.79	4.40 ± 1.14	9.40 ± 4.39 (12)	3.60 ± 1.67(14)	6.20 ± 1.10 (9)	0.20 ± 0.45 (1)	0.00 ± 0.00 (0)
	R	91	2.09	11.20 ± 7.09	27.00 ± 9.54 (15)	9.60 ± 6.39(17)	4.20 ± 2.95 (6)	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)
Overall abundance				*17.78 ± 6.15*[a]	*11.17 ± 4.3*[a,b]	*8.65 ± 4.19*[b]	*9.13 ± 3.08*[b]	*0.13 ± 0.21*[c]	*0.16 ± 0.22*[c]
Total number of species				-	61	78	33	5	5

† Indicates insect groups that differed significantly from all other groups sampled in 2009. ‡ Indicates insect groups sampled in 2009 where no significant differences occurred between the groups. a-c Indicate significant differences between insect groups sampled in 2010.

(Approximative Wilcoxon Mann-Whitney rank-sum tests, $P < 0.05$)

Table 2.2: Relationships between insect order abundance and species richness with percent natural habitat. Normally distributed variables (N) were analysed using simple linear regression and variables with poisson distributions (P) were analysed using generalized linear regression. Response variables (V) were transformed (Trans) to normality where possible.

	Abundance					Species richness				
Insect group	Trans	Dist	β	F	R^2	Trans	Dist	β	F	R^2
2009										
non-*Apis* Hymenoptera	sqrt(V)	N	0.005	1.30	0.04	sqrt(V)	N	-0.000	0.01	0.00
Diptera	log(V)	N	-0.013	56.43***	0.63	log(V)	N	-0.007	36.62***	0.53
Coleoptera	sqrt(V)	N	-0.010	4.52*	0.12	sqrt(V)	N	-0.002	0.44	0.01
Hemiptera	sqrt(V)	N	-0.003	0.53	0.02	sqrt(V)	N	-0.001	0.10	0.00
Lepidoptera	sqrt(V)	N	-0.012	9.48**	0.22	sqrt(V)	N	-0.010	7.61**	0.19
2010										
non-*Apis* Hymenoptera	sqrt(V)	N	-0.011	4.55*	0.08	V	N	-0.003	0.05	0.00
Diptera	sqrt(V)	N	0.014	3.48	0.06	V	N	-0.012	1.72	0.03
Coleoptera	sqrt(V)	N	-0.014	12.28***	0.19	log(V)	N	-0.005	27.01***	0.34
				Z	AIC				Z	AIC
Hemiptera	V	P	-0.021	-1.87	45.1	V	P	-0.021	-1.87	45.1
Lepidoptera	V	P	-0.064	-3.76***	31.2	V	P	-0.059	-3.42***	27.2

(***$P < 0.001$, **$P < 0.01$, *$P < 0.05$), N = normal distribution, P = Poisson distribution

Table 2.3: Results of the distance-based redundancy analyses (dbRDA) for 2009 and 2010 pan trap data. The best models for both years included all explanatory variables.

Year	Predictor variable	Proportion of total variability explained	*P*	Pseudo-F
2009	Field health	25.6%	0.0001	11.37
	Method of irrigation	10.7%	0.0001	5.38
	Managed hive stocking density (/ha)	7.3%	0.0001	4.01
	Natural habitat (%)	5.5%	0.0001	3.22
	Wind speed (km/h)	5.9%	0.0001	3.81
	Field size (ha)	6.3%	0.0001	4.54
	Ambient temperature (˚C)	2.3%	1	0
2010	Natural habitat (%)	12.1%	0.0001	7.29
	Method of irrigation	12.1%	0.0001	4.07
	Wind speed (km/h)	5.6%	0.0001	4.00
	Field health	6.3%	0.0001	4.80
	Field size (ha)	3.7%	0.0003	2.93
	Managed hive stocking density	4.2%	0.0001	3.54
	Ambient temperature (˚C)	2.5%	0.0061	2.12

2009 model: AIC = 245.66, R^2 = 0.61, df = 7 and 2010 model: AIC = 406.85, R^2 = 0.46, df = 9

Table 2.4: The flower-visiting species sampled in pan traps in 2009 and 2010 with multiple partial correlations > 0.2 for the first two canonical axes of the dbRDA ordinations.

Year	Species	Family	dbRDA1	dbRDA2	Characterizing sites with
2009	*Halictus* spp.	Halictidae	-	-0.54	Drip irrigation
	Anthomyiidae sp.2	Anthomyiidae	0.22	-0.23	Low natural habitat (%) Sub-standard field health
	Diptera sp.29		-	-0.22	
	Limnophora quaterna	Muscidae	0.47	-	
	Calyptratae sp.2		0.31	-	
	Chrysomya albiceps	Calliphoridae	0.36	-	
	Chrysomya chloropyga	Calliphoridae	0.21	-	
	Musca spp.	Muscidae	0.47	0.23	
	Muscidae sp.1	Muscidae	0.24	0.27	
	Hemiptera sp.6		-	0.27	
2010	*A.m. capensis*	Apidae	-0.43	-	Low natural habitat (%)
	Neomyia peronii	Muscidae	-0.33	-	
	Lasioglossum sp.14 & 15	Halictidae	-0.39	-	
	Pteraulax sp.	Bombyliidae	-0.24	-	
	Buprestidae sp.1		-0.16	-0.17	
	Hyperusia sp.	Bombyliidae	0.31	0.27	High natural habitat (%) Drip irrigation
	Chrysomya chloropyga	Calliphoridae	-	0.29	
	Lasioglossum sp.1	Halictidae	-	0.28	
	Ceroctis aliena	Meloidae	0.14	-0.16	High natural habitat (%) Sprinkle and flood irrigation
	Lioadalia flavomaculata	Coccinellidae	-	-0.22	
	Hippodamia variegata	Coccinellidae	-	-0.36	

Table 2.5: Results of the generalized linear mixed model analyses of the abundance of honeybees sampled in pan traps in 2009 and 2010 for the 18 hybrid onion field sites. The Z-values from likelihood ratio tests and the direction of the relationships are shown for the variables in the two final models for each year. Blank spaces represent dropped variables following stepwise deletion. Number of observations = 35, for 7 farms for the 2009 models, and 55, for 11 farms for the 2010 models .

Model	AIC	SD$	Natural habitat (%)	Hive density (/ha)	Temperature (˚C)	Wind speed (km/h)	Field size (ha)	Method of Irrigation†	Field Health‡
2009 M1	70.85	0.18			-2.01* (-)	-2.07* (-)		1.42 s > d	
2009 M2	72.26	0.17		-0.79 (-)	-2.04* (-)	-2.29* (-)		1.64 s > d	
2010 M1	177.3	0.57		-2.29* (-)	-1.3 (-)		-1.87 (-)	-0.25 d > f 1.88 d < s	
2010 M2	179.2	0.56	-0.31 (-)	-2.01* (-)	-1.02 (-)		-1.53 (-)	-0.20 d > f 1.48 d < s	

(***$P < 0.001$, **$P < 0.01$, *$P < 0.05$), $ Standard deviation of random factor. † Method of irrigation included drip (d) and sprinkle (s) in 2009 and drip (d), sprinkle (s) and flood (f) in 2010, ‡ Fields were scored as healthy (h) or sub-standard (s).

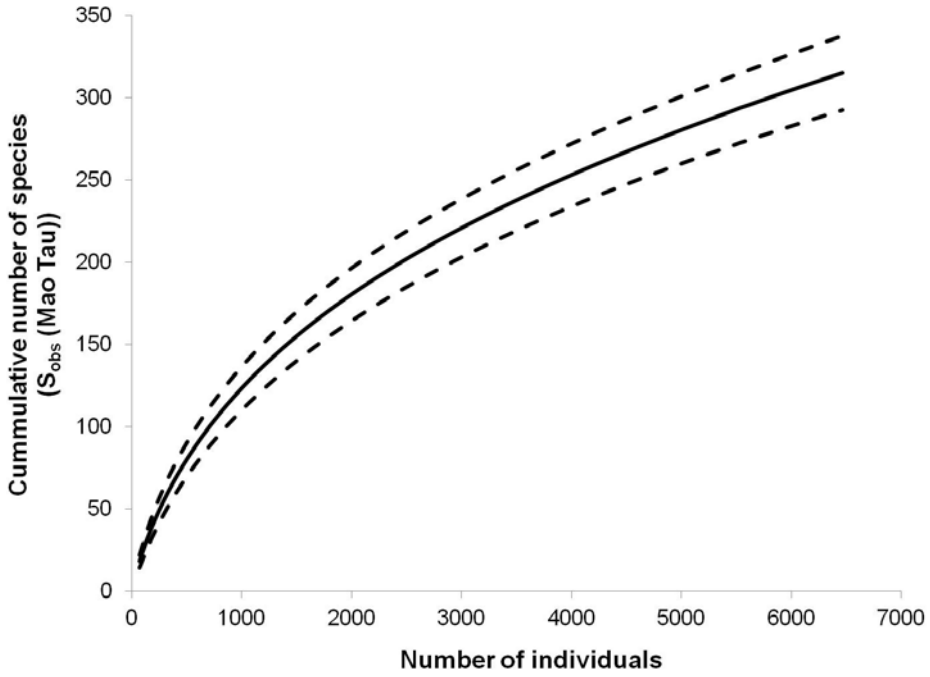


Figure 2.1: Sample-based rarefaction curve (solid black line with 95% upper and lower bound confidence intervals as dotted lines) for all pan trap samples.

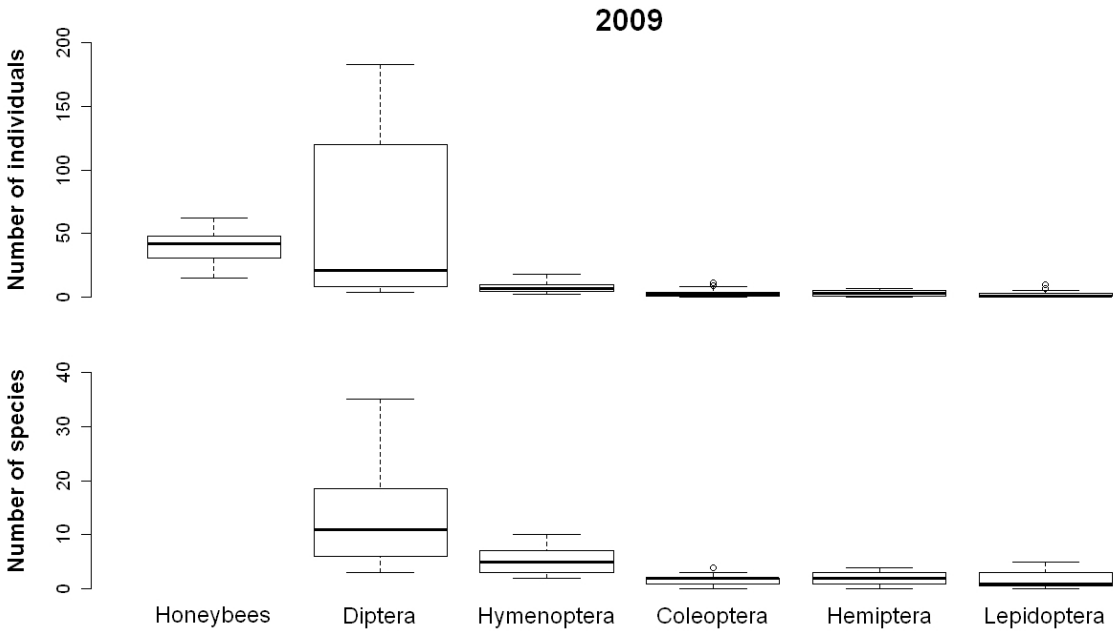


Figure 2.2: Honeybee abundance and insect order abundance and species richness sampled with pan traps in 2009. (median with lower and upper quartile and non-outlier range, open circles indicate outliers) (see Table 2.1 for statistics)

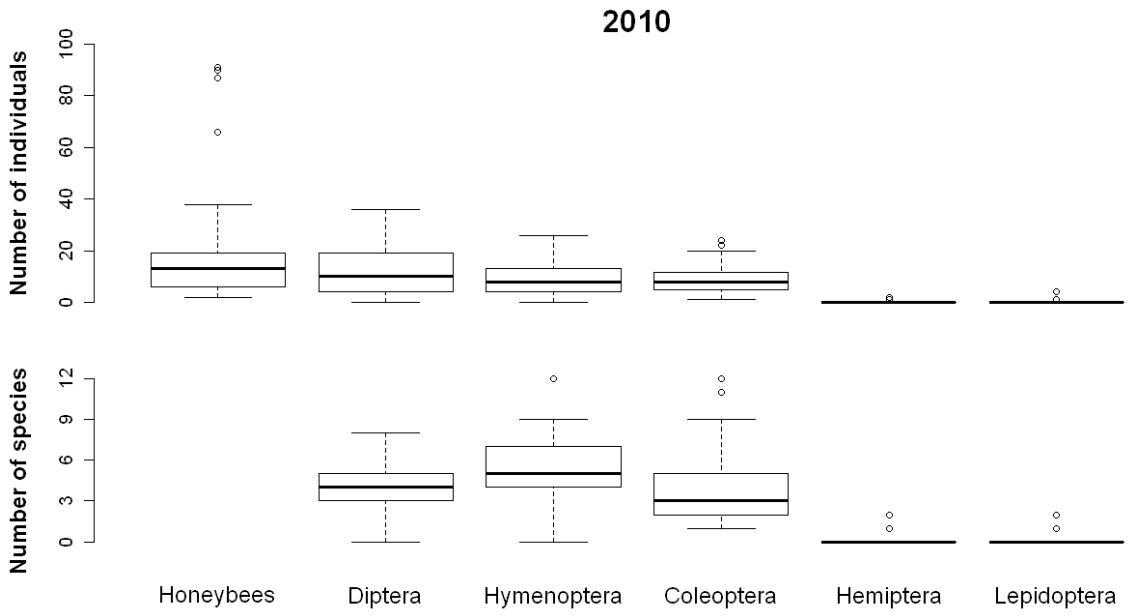


Figure 2.3: Honeybee abundance and insect order abundance and species richness sampled with pan traps in 2010. (median with lower and upper quartile and non-outlier range, open circles indicate outliers) (see Table 2.1 for statistics)

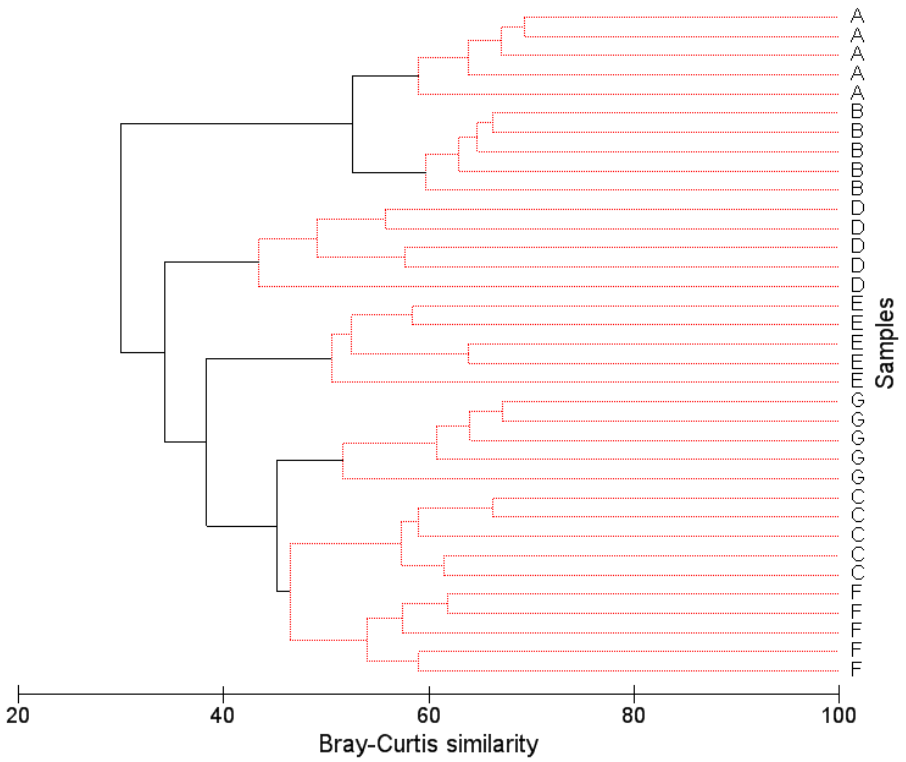


Figure 2.4: The dendogram of a hierarchical cluster analysis of the 2009 pan trap samples from each of the 7 sites (A to G). The grey lines indicate where the SIMPROF tests did not find significant differences between the samples.

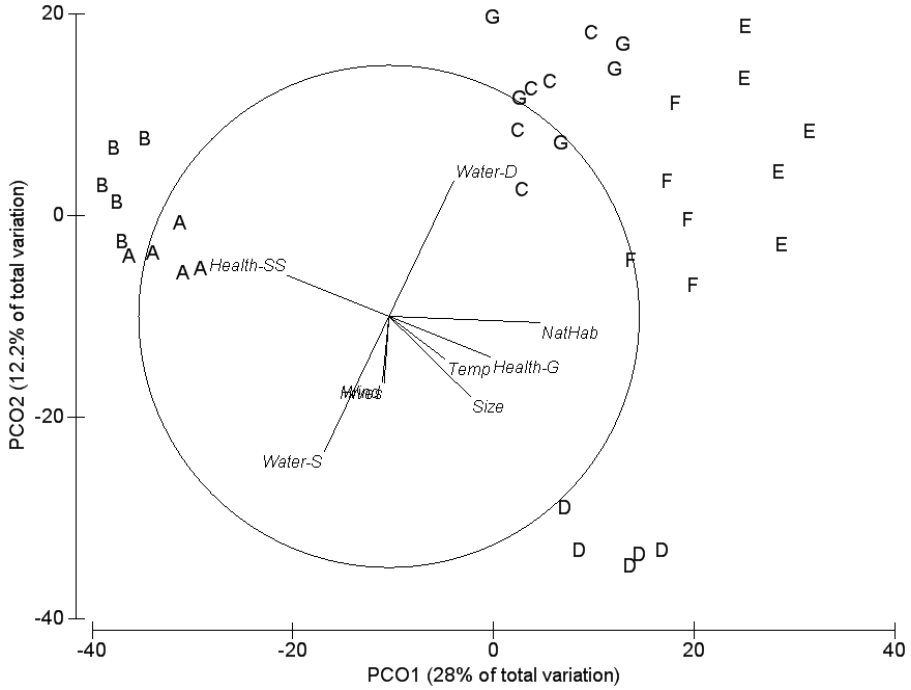


Figure 2.5: An unconstrained ordination of a principal coordinates analysis (PCO) of the 2009 pan trap samples. The letters denote the samples taken at each of the seven sites. The vector overlay depicts multiple partial correlations of the explanatory variables to the two PCO axes.

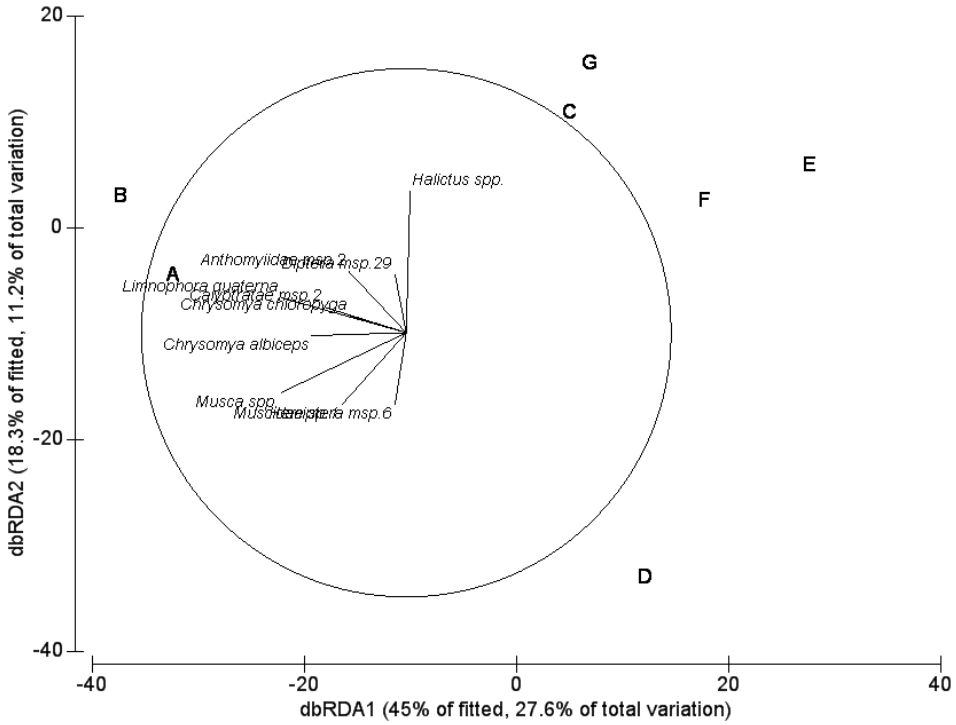


Figure 2.6: A constrained ordination of the first two axes of the distance-based redundancy analysis for the 2009 pan trap data. The vector overlay depicts species variables with multiple partial correlations greater than 0.2 with the two dbRDA axes. The letters denote the seven sites. The identified species are listed in Table 2.4.

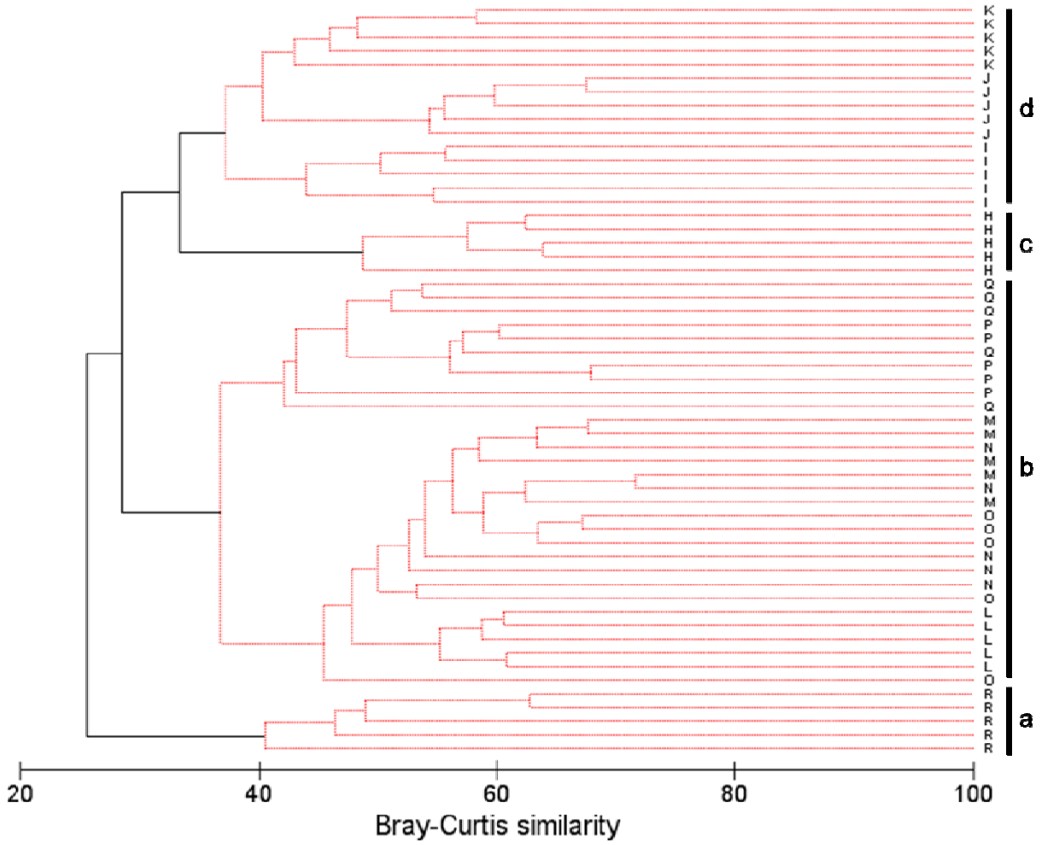


Figure 2.7: The dendogram of a hierarchical cluster analysis of the 2010 pan trap samples at each of the 11 sites (H to R). The grey lines indicate where the SIMPROF tests did not find significant differences between the samples.

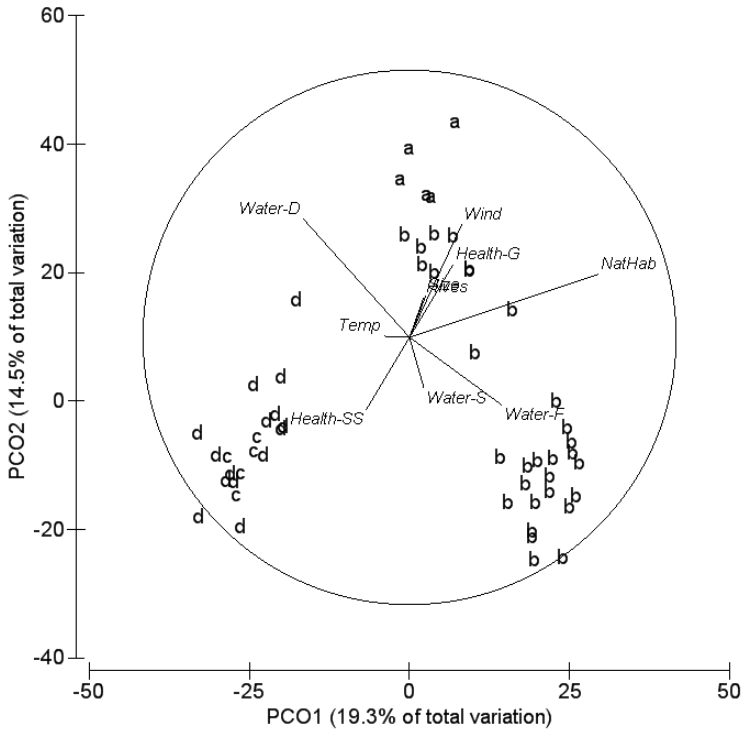


Figure 2.8: An unconstrained ordination of a principal coordinates analysis (PCO) based on a Bray-Curtis resemblance matrix of the 2010 pan trap samples. The letters denote the samples of to the 4 groups identified by the cluster analysis.

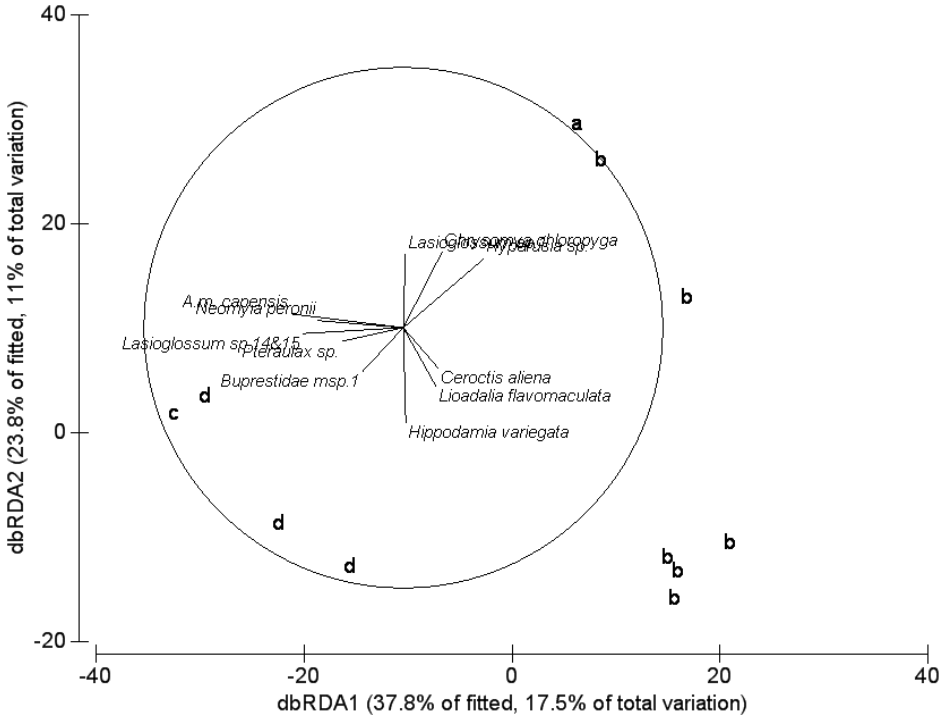


Figure 2.9: A constrained ordination of the first two axes of the distance-based redundancy analysis for the 2010 pan trap data. The vector overlay depicts species variables with multiple partial correlations greater than 0.2 with the two dbRDA axes. The letters denote sites belonging to the 4 groups identified by the cluster analysis. The identified species are listed in Table 2.4.

3

WHICH INSECT GROUPS ARE EFFECTIVE POLLINATORS OF ONION HYBRID SEED CROPS IN SOUTH AFRICA?

ABSTRACT

Insect visitation is essential in the pollination of onion hybrid seed crops. Pollinators carry pollen from the pollen producing male-fertile parent plants to the seed-producing male-sterile plants which produces no pollen through genetic inhibition. The honeybee, *Apis mellifera* Esch., is widely used to pollinate hybrid onion crops. However, many other insect species have been documented as visitors to hybrid onion flowers. In some cases, certain non-*Apis* bee species are more efficient as onion pollinators. In this chapter, I assess the abundance (visitation frequency), visitation rates (number of florets visited) and the pollen loads, among other variables, of various insect visitors recorded on hybrid onion umbels to determine which insect visitors would be most likely to be the main pollinators of onion hybrid seed crops. I also determined whether this crop is receiving pollination ecosystem services from non-*Apis* species, and to what extent. My results indicated that the honeybee was by far the most abundant visitor on the umbels, and individuals carried significant loads of onion pollen on their bodies. Much less abundant insect visitors (< 5%) were ladybirds, flies and milkweed bugs, while non-*Apis* bees, wasps, butterflies and other beetles only rarely visited the umbels. Of the three most abundant non-*Apis* insect groups assessed for pollen loads, milkweed bugs (the least abundant) carried the most pollen, followed by calliphorid flies (second most abundant), while ladybirds (the most abundant of the three) carried the least pollen. Based on these findings, I conclude that honeybees are the main pollinator of onion hybrid seed crops in South Africa through high visitation frequencies and significant onion pollen loads. However, no clear distinction could be made between managed pollination services and pollination ecosystem services delivered by wild honeybees. Annual rainfall had a significant effect on honeybee visitation frequency; a factor of high importance for resident managed and wild honeybees reliant on floristic resources that are determined by annual rainfall. The significant positive relationship between the diversity of hand-sampled insects and honeybees observed foraging on onion flowers; indicate that either or both varietal attractiveness and/or pollinator population size might have had significant effects on overall insect visitation.

INTRODUCTION

F1 hybrid onion cultivars have been developed following the discovery of cytoplasmic male sterility in the onion by Jones & Clarke (1943). Cross-breeding parental lines have been established where pollen producing male-fertile plants are planted in rows next to seed-producing male-sterile plants at ratios varying mostly between 1:3 and 1:8 (George, 1999). Onion hybrid seed production is entirely dependent on insects as pollen vectors to carry pollen from the male-fertile to the male-sterile flowers in order to bring about successful pollination (Chandel, *et al.*, 2004; McGregor, 1976; Munawar *et al.*, 2011).

Onion (*Allium cepa* L.) inflorescences carry between 200 to 600 individual florets, depending on the cultivar (Brewster, 2008). Individual onion florets are only 3 to 4 mm in length and are open to cup-shaped with the reproductive parts fully exposed (see Figure 1.1). The onion therefore has a generalized pollination syndrome where wide varieties of different insects visit the flowers and have the potential to pollinate them. In general, the factors that determine whether an insect is an effective pollinator are: its abundance on the flowers; its rate of visiting the flowers; whether it touches the floral reproductive parts; and whether it carries sufficient loads of conspecific pollen on its body. Several authors have investigated the efficiency of mostly Diptera and Hymenoptera flower-visitors as pollinators of hybrid onion inflorescence (Currah & Ockendon, 1983, 1984; Dowker *et al.*, 1985; Moffett, 1965; Parker, 1982; Saeed *et al.*, 2008; Sajjad *et al.*, 2008; Schittenhelm *et al.*, 1997; Waller *et al.*, 1985; Walsh, 1965; Williams & Free, 1974; Witter & Blochtein, 2003). In controlled experiments using large cages, Currah & Ockendon (1983, 1984) found no difference in pollination efficiency between honeybees and three species of blowflies (Calliphoridae) in terms of seed set and crossing rate between two onion cultivars. However, Dowker *et al.* (1985) found that yields of onion hybrid seed were much higher for honeybee pollination than for blowfly pollination. Similarly, Moffett (1965) and Walsh (1965) found that houseflies (Muscidae) were considerably less efficient than honeybees in pollinating onion flowers in caged experiments. In field experiments, Saeed *et al.* (2008) assessed the pollination potential of true flies and native bees in Pakistan and found that bees showed significantly greater pollination effectiveness than flies in that a single visit by a bee species produced more seed per umbel than a single visit by a fly, mostly syrphids.

Although the onion is self-compatible, self-pollination is naturally limited by individual onion florets being protandrous, the anthers dehisce before the stigma becomes receptive

(Muller, 1883). Self-pollination is totally prevented when producing hybrid seed (Williams and Free, 1974). When the anthers of onion florets dehisce, all the pollen is shed within 2 to 3 days, but most of the pollen is shed on the first day (McGregor, 1976). Onion pollen is equally viable whether collected early or later in the day, but the viability rapidly declines after the first day subsequent to anthesis (Mann & Woodbury, 1969). The stigma of the onion floret stays receptive for about 3 days, after which receptivity gradually decreases until it ceases entirely after 6 to 7 days (Moll, 1954). Consequently, pollen needs to be transported on the day of anthesis for optimal germination potential, while viable pollen has to reach the stigma within the first 3 days of receptivity. When thousands of hybrid onion umbels come into flowering, an abundance of pollinators are needed to ensure extensive and continuous crop pollination during the entire blooming period (Nye *et al.*, 1971). South African seed companies implement strict quality controls and regulations to ensure that seed growers produce the required quantity as well as quality of onion hybrid seed. Seed germination should be above 80% in post-harvest trails, or else seed companies would not buy the harvest from the farmers. Therefore, adequate pollination is essential for onion hybrid seed growers to produce a marketable crop.

Aims

The central aim of this chapter is to identify the insect visitors that are likely to be the main pollinators of onion hybrid seed crops in South Africa, by considering visitation frequencies and foraging behaviour on male-fertile and male-sterile umbels, and assessing pollen loads from the bodies of frequent insect visitors. The previous chapter had shown high anthophile diversity within onion crop fields and its relationship to environmental (e.g. percentage natural vegetation) and management practices (e.g. watering regimes). However, the applicability of this diversity in terms of its pollination efficiency is not certain and how this diversity contrasts to the efficiency of honeybee pollination is also not known. The metrics used in this chapter should allow me to determine the key pollinator species found within this general anthophile diversity associated with onion hybrid seed pollination.

METHODS

Study Sites

Data was collected from 13 healthy onion hybrid seed crops (5 farms in 2009 (C-G) and 8 farms in 2010 (I-M, P-R)) grown in the Klein Karoo and southern Karoo regions in the Western Cape (refer to Table A.1, and Figure A.1 in Appendix A). A crop was considered "healthy" if there were no obvious signs of disease in at least 80% of the plants at the time of data collection. The climatic characteristics of the Klein Karoo and southern Karoo are optimal for onion hybrid seed production that requires low humidity and mild cool temperatures during the initial growth phase, followed by increased temperatures later on to induce flowering and support pollinator activity (Shanmugasundaram, 1998). The main vegetation type in the low-lying areas of both regions is Succulent Karoo vegetation which is characterized by open to sparse dwarf (up to 1 m tall) shrublands dominated by stem and leaf succulents and some fine-leaved evergreen shrubs (Hilton-Tailor and Le Roux, 1989). The Succulent Karoo biome is recognised as a global biodiversity hotspot with exceptionally high levels of faunal and floral species richness and endemism (Myers *et al.*, 2000). For further details on the biogeographic characteristics, please refer to Chapter 2. Varietal differences between the farms were based solely on coded information given by the participating seed companies, due to the sensitive nature of hybrid onion breeding information (see Table A.1).

Climatic variables on days of sampling

Data was collected during peak flowering (>50% florets open) during late October and November (2009-2010) on sunny days with optimal weather conditions for anthophile activity. Ambient temperatures (˚C) and wind speeds (km/hour) were recorded throughout the data sampling day using a hand held weather tracker (Kestrel 4000, Nielsen-Kellerman U.S.A.). Mean daily temperatures and wind speeds were calculated for each sampling day. Data for daily rainfall (mm) were obtained from the South African Weather Service (www.weathersa.co.za), measured daily during 2009 and 2010 for two locations within the study area (Laingsburg Municipality (Ref. nr. 0045611 7), De Rust Police Station (Ref. nr. 0049060 8)). The total annual rainfall recorded at the nearest station to each field, within the respective year, was used for analyses.

Foraging activity of insect visitors

Flower-visitors were observed for one good-weather day on each crop field. Observations were made during four data collection periods spread over 2 hour intervals between 09h00 and 16h00. Observations were replicated five times on both male-fertile and male-sterile rows during each collection period. Four neighbouring umbels in at least 50% bloom were selected and observed for 4 minutes. The number and identity of visitors to the umbels (visit frequency) were recorded. Visitors were later grouped into visitor guilds (*A.m. capensis*, non-*Apis* bees, Diptera, Coccinellidae, Hemiptera, wasps, butterflies and other Coleoptera). Data was also collected on the approximate number of florets visited by each insect, the floral resource (nectar and/or pollen) that was collected by each visitor and if the insect touched the stigmas and/or the anthers of the florets. During each data collection period, 15 minutes were spent on each parental line collecting insects that foraged on the umbels, using hand and butterfly nets. This data was used for species identification and quantifying the diversity of insects foraging on the umbels. Reference collections of morphospecies were sent to various expert taxonomists for identification and were later deposited in museum collections (a list of the identified taxa appears in Appendix B).

Pollen loads on the bodies of foraging insects

To quantify the pollen loads on the bodies of foraging insects, insect visitors were sampled from male-fertile and male-sterile rows using hand and butterfly nets and were then stored separately in marked 1.5 ml Eppendorf tubes filled with 70% ethanol. Care was taken to avoid catching honeybees with pollen gathered in their corbicula so that only the pollen loads on the insect's body could be assessed, as this is the pollen that is available for pollination. Pollen loads were quantified only for the four most abundant species recorded on the umbels. The low numbers of sampled specimens of all other insect species would not allow for meaningful statistical results and were thus excluded. The procedure described in Kendall & Solomon (1973) was followed to dislodge and count the pollen from the body of each insect. The tube with the single specimen was shaken for one minute and then emptied and rinsed with 70% ethanol in a small flat-bottomed transparent plastic container with a diameter of 41 mm. The body of the insect was held in the liquid in the dish with forceps and thoroughly brushed with a fine painters brush to dislodge all pollen from its body. The insect was then sprayed with a fine jet of 70% ethanol while being held over the dish. A drop of Calberla's fluid (Dafni *et al.*, 2005) was added to the mixture to stain the pollen grains. The liquid was

then shaken to distribute both the stain and pollen evenly, and then left for 10 minutes to allow the pollen grains to be stained and eventually settle to the bottom of the container.

Seventeen equal sized and evenly spread black dots with 1 mm diameter were made on a disk of white paper with the same diameter as the bottom of the transparent dish containing the pollen loaded liquid (Kendall and Solomon, 1973). The paper disk was then fixed to the bottom of the container. The dots covered approximately 1% of the total surface area. All the pollen grains within each of the black dots were counted using a stereo-microscope with 40x magnification (Wild Heerbrugg M3C, Switzerland). The total number of pollen grains were estimated by multiplying the average number of pollen grains counted in each dot by 1,681.85 (total area of the container divided by the area covered by one dot). The small size of the black dots allowed light to be reflected so that the pollen grains within each dot were visible but no distinction could be made between pollen grains from different plant species.

In order to calculate the percentage of onion pollen versus other pollen species, a drop of liquid was sampled from the container and placed on a microscope slide and then left so that the excess alcohol could evaporate. A drop of glycerine was added and the sample was then covered with a cover slide. An upright microscope with 100x magnification (Carl Zeiss Axio Scope, Germany) was used to count all the pollen grains under the cover slide of the microscope slide. Reference slides of hybrid onion pollen were made in the field by rubbing dehisced stamens on a microscope slide. This allowed the sub-sample of grains to be scored as being 'onion' or 'other' pollen grains. Finally, this data was then used to calculate the percentage of onion pollen grains as opposed to other pollen grains on the bodies of insect foragers on hybrid onion umbels.

Data Analysis

Foraging activity of insect visitors

Non-parametric approximative Wilcoxon Mann-Whitney (WMW) rank-sum tests (linked with Monte-Carlo re-sampling to compute the null distribution of the test statistic) were used to test for significant differences between pairs of samples ($P < 0.05$), using function 'wilcox_test' from the '*coin*' package (Hothorn *et al.*, 2008) in R v.2.14.1, (R Development Core Team, 2011). Differences in visitation frequency on male-fertile and male-sterile umbels were tested for the 5 most abundant visitor groups. Foraging behaviour is reported as

percentages, and focuses on the collection of floral resources, contact with reproductive floral parts, and the numbers of florets visited.

Pollen loads on the bodies of foraging insects

Differences in pollen loads were tested with approximative WMW rank-sum tests using a significance level of $P < 0.05$. Pollen loads of insects sampled from male-fertile and male-sterile umbels were tested within each visitor group; while differences between groups were also tested. The identity of pollen grains carried on the bodies of hybrid onion umbel foragers are reported as percentage onion versus other pollen grains.

Factors that influence honeybee visitation frequency

A generalized linear mixed-effects model (GLMM), fitted by the Laplace approximation, with a Poisson error distribution and log link function was used to test the effects of different explanatory variables on honeybee visitation frequency. The explanatory variables used as fixed terms in the model included the following variables recorded and estimated for each site: the percentage natural habitat that surrounded each site within a 500 m radius (see Chapter 2 for details); the stocking density of managed honeybee hives (see Chapter 2 for details); hybrid onion crop field size; total annual rainfall; mean daily temperature and mean daily wind speed. Spearman-rank correlation tests were used to test for collinearity between the continuous fixed variables with $r_S \geq 0.7$ used to indicate strong collinearity (Zuur *et al.*, 2009).

GLMM models correct for over-dispersion by allowing the use of a random effect in the model (Zuur *et al.*, 2009). The sampling sites were used as random effect. R v.2.14.1 statistical software was used (R Development Core Team, 2011) with the "lmer" function from the "lme4" package (Bates *et al.*, 2011). Backward elimination selection procedures were followed for model simplification, starting with a model including all explanatory variables as fixed terms and dropping the least significant term, or the term which resulted in a lower AIC value, after contrasting the reduced model to the full model using ANOVA. If dropping a term did not result in a significant change ($P < 0.05$) in the model, the reduced model was retained.

RESULTS

Foraging activity of insect visitors

Honeybees were the most frequent visitors on both male-fertile and male-sterile hybrid onion umbels during each of the four daily recording bouts (Table 3.1, Figure 3.1), with a total of 2,817 individuals recorded (91.5% of total number of visitors). Ladybird beetles (Coleoptera: Coccinellidae) and flies (Diptera) were less frequent visitors, with 139 (4.5%) and 84 (2.7%) individuals recorded, respectively (Table 3.1). Bugs (Hemiptera), non-*Apis* bees and wasps (Hymenoptera), butterflies (Lepidoptera) and other beetles (Coleoptera) were present on the umbels in very low frequencies (1.3% of visits collectively). A significant, positive relationship between honeybee visitation frequency and the diversity of hand-sampled insects foraging on the umbels was recorded ($r_s = 0.80$, Figure 3.2). (Lists of species that were foraging on the umbels are listed in Appendix B under hand-collected specimens).

Honeybee visitation frequency differed across field sites on both male-fertile and male-sterile umbels (Figure 3.3). Generally, sites sampled in 2010 (I-R) had lower honeybee visitation frequencies (2.05 ± 2.47) than sites sampled in 2009 (C-G) (10.81 ± 5.90). Even though Coccinellidae visitation frequency was higher at sites where honeybee visitation frequency was lower (Coccinellidae: 0.79 ± 0.53 vs. *A. m. capensis*: 1.48 ± 1.72), the visitation frequency of Coccinellidae was still very low (Figure 3.4).

Significantly more honeybees were counted on male-fertile umbels than on male-sterile umbels ($Z_{1, 259} = 4.82$, $P < 0.05$) and the same was recorded for Hemiptera ($Z_{1, 259} = 2.44$, $P = 0.02$) (see Table 3.1). There was no significant difference in the visitation frequency of Coccinellidae ($Z_{1, 259} = -1.69$, $P = 0.11$), Diptera ($Z_{1, 259} = -1.37$, $P = 0.21$), or non-*Apis* bees ($Z_{1, 259} = 0.31$, $P = 1$) on male-fertile and male-sterile umbels. Honeybees, flies and ladybirds were more numerous on the umbels later in the day than earlier in the day, while the numbers of Hemipteran foragers were lowest during the hottest time of the day (Figure 3.5).

Of the four most abundant insect groups, visitors mostly collected nectar on male-fertile umbels with very few pollen collectors recorded (Table 3.2). Of the honeybee foragers, 2.6% of honeybees foraging on male-fertile umbels collected pollen only, while 25.5% collected nectar and pollen, and the remaining 71.7% collected nectar only ($N = 572$). Most insect visitors touched both the male and female reproductive parts of florets during foraging on male-fertile and male-sterile umbels (Table 3.2). 60% of honeybee foragers visited less than 10 florets on male-sterile umbels (Table 3.2). The number of male-sterile florets visited by

Coccinellidae and Diptera varied, while most Hemiptera foragers visited more than ten male-sterile florets per foraging bout (83.3%).

Pollen loads on the bodies of foraging insects

Honeybees carried the most pollen grains on their bodies (onion and other pollen), followed by the milkweed bug, *Spilostethus pandurus* Scopoli (Hemiptera: Lygaeidae) (Table 3.3, Figure 3.6). There was no significant difference in the pollen loads carried by individuals of these two insect species foraging on male-sterile umbels ($Z_{1, 53} = 1.41$, $P = 0.17$), although the pollen loads on individuals that foraged on male-fertile umbels differed significantly ($Z_{1, 43} = 4.26$, $P < 0.001$). Calliphorid flies and ladybird beetles of the alien species *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) that foraged on male-fertile and male-sterile umbels carried significantly less pollen grains than honeybees and *S. pandurus* (male-fertile umbels: calliphorid flies vs. honeybees: $Z_{1, 46} = -5.34$, $P < 0.001$; calliphorid flies vs. *S. pandurus*: $Z_{1, 28} = -2.46$, $P = 0.01$; *H. variegata* vs. honeybees: $Z_{1, 47} = -5.68$, $P < 0.001$; *H. variegata* vs. *S. pandurus*: $Z_{1, 29} = -3.79$, $P < 0.001$; male-sterile umbels: calliphorid flies vs. honeybees: $Z_{1, 60} = -4.18$, $P < 0.001$; calliphorid flies vs. *S. pandurus*: $Z_{1, 22} = -2.26$, $P = 0.03$; *H. variegata* vs. honeybees: $Z_{1, 66} = -8.23$, $P < 0.001$; *H. variegata* vs. *S. pandurus*: $Z_{1, 28} = -3.81$, $P < 0.001$). Honeybee individuals that foraged on male-sterile umbels carried significantly less pollen grains than those that foraged on male-fertile umbels ($Z_{1, 76} = -5.33$, $P < 0.05$), while the same was found for *H. variegata* foragers ($Z_{1, 30} = -3.65$, $P < 0.05$) (Figure 3.6). Over 90% of the pollen grains carried on the bodies of honeybees and calliphorid fly foragers on male-fertile and male-sterile umbels were onion pollen (Table 3.3). High percentages of onion pollen grains were also recorded from *H. variegata* and *S. pandurus* foragers on both parental lines (see Table 3.3).

Factors that influence honeybee visitation frequency

There was a significant collinearity between percentage natural habitat and total annual rainfall (Table 3.4). The model, where either percentage natural habitat, or total annual was dropped, which had the lowest AIC value were chosen for further analysed. Most factors were not significantly contributing to the observed patterns and dropped out of the model. These included daily temperature; daily wind speed; crop field size and method of irrigation. The model was improved by retaining the factor managed hive stocking density, but it had no significant effect on honeybee visitation frequency. The only significant factor was total

annual rainfall which had a positive effect on honeybee visitation frequency (Table 3.5). High honeybee visitation frequencies were recorded in 2009 with total annual rainfall recorded in the two regions being 424.6 mm, (De Rust) and 246.6 mm (Laingsburg), while considerably fewer honeybees visited the crops in 2010 with total annual rainfall of 234.8 mm and 96.8 mm being recorded at De Rust and Laingsburg stations respectively.

DISCUSSION

Foraging activity of insect visitors

A small percentage (<20%) of the anthophile diversity that was recorded within onion hybrid seed crops in chapter 2, actually visited the umbels, representing less than 10% of all visits. My findings are similar to those of many others and confirm that honeybees are often the most frequent, and in some cases, the only pollinating agent visiting blooming onion crops in economically viable numbers (Caron *et al.*, 1975; Free, 1970; Williams & Free, 1974). Some studies have, however, reported non-*Apis* visitors as efficient pollinators of onion flowers. Parker (1982) reported that a native North American bee, *Halictus farinosus* Smith, was the most abundant forager recorded on the umbels of an experimental plot of hybrid onions. Lederhouse *et al.* (1968) did hand-collections of insects foraging on commercial flowering onion crops in New York State and found that of the 1,172 insects collected, 52% were wild bees while flies accounted for 35%, together with Hemiptera (6%), Coleoptera (4%), and Lepidoptera (1%) that were also represented in their sample. Saeed *et al.* (2008) found that Diptera accounted for 87% of foragers on a blooming onion crop in Pakistan. In stark contrast, Dipteran foragers in my study accounted for only 2.7%, and non-*Apis* bees only for 0.4% of the total number of foragers recorded on umbels. Of the Diptera recorded, carrion flies were the most frequent visitors (54%), while syrphids accounted for only 7% of visits. This latter finding further contrasts with the findings of Saeed *et al.* (2008), who found Syrphidae to be the most abundant Dipteran visitor (74% of total Dipteran visitors).

Even though the visitation frequency of non-*Apis* insects did not compare to that of honeybees, the high correlation between non-*Apis* diversity and honeybee visitation frequency indicates the potential importance of two factors that may determine insect visitation to onion hybrid seed crops; (i). the overall attractiveness of the different crops (varieties) to insect foragers; and (ii) the size of the anthophile population present during a particular time and space, that could visit the hybrid onion flowers. Spatial and temporal

shifts in pollinator diversity and population size are highly variable (Roubik, 2001; Williams *et al.*, 2001), and may be influenced by various ecological factors like the availability of floral resources and suitable nesting sites.

Interestingly, and in comparison to other onion pollination studies done in other parts of the world (see above references), ladybird beetles were the most frequent non-*Apis* visitor observed on the umbels, and also were frequently observed to imbibe nectar from the florets. Even though ladybird beetles are mainly aphidophagous predators, their diet can also extend to non-prey foods, such as nectar and pollen (Lundgren, 2009). It has also been found that the reproductive performance of ladybirds (*H. variegata* in particular) increased when individuals were provided with plant species that offered pollen and nectar as supplementary food sources (Bertolaccini *et al.*, 2008), thus indicating the importance of floral resources for ladybirds. Other unexpected visitors observed on the umbels were species of true bugs (Hemiptera), which were also observed feeding on nectar. Similarly, Howlett *et al.*, (2005) reported the observation of Heteroptera visitors in contact with onion flowers in New Zealand crops, while Lederhouse *et al.* (1968) collected Hemiptera from onion umbels in New York. Armstrong (1979) reported sightings of nectar-seeking Lygaeidae (Hemiptera) on two Australian plant species, and members of the family Miridae (Hemiptera) as flower-visitors on another two Australian plant species. These findings confirm hemipteran visitors as flower-visitors seeking floral resources on occasion.

Even though honeybee visitation frequency was significantly higher on male-fertile than on male-sterile umbels, a considerable number of honeybees were still present on male-sterile umbels, which contrasts with the findings of Lederhouse *et al.* (1972) who found that the honeybee population was almost three times greater on male-fertile umbels. The honeybee population foraging on the male-sterile umbels investigated here could therefore have been satisfactory for adequate pollination levels to occur. The fact that Coccinellidae and Hemiptera were present on the umbels validates the possibility for these individuals as onion pollen vectors. However, the mere presence of an insect on a flower does not guarantee cross-pollination (Dafni *et al.*, 2005). The foraging behaviour of the two insect groups suggests that they were active nectar foragers, while touching both the anthers and the stigmas of the florets, thus indicating their potential as pollinators.

Pollen loads on the bodies of foraging insects

Honeybees that foraged on male-fertile umbels carried the most pollen grains on their bodies in comparison to the other insects investigated. This is in accordance with other findings that generally show that the bodies of honeybees carry more pollen than other insect visitors (Kendall and Solomon, 1970; Free and Williams, 1972). The body hairs of bees are branched and densely packed and are thus adapted to easily accumulate pollen (Thorp, 1979). This pollen is readily available to pollinate receptive stigmas. Williams & Free (1974) noted that although honeybees do not favour onion flowers as a pollen source, their bodies become dusted with pollen when they forage for nectar on the male-fertile umbels. However, Parker & Hatley (1979) assessed the viability of pollen grains on the bodies of pollinator insects and found that pollen on the bodies of honeybees were less viable than the pollen on the bodies of non-*Apis* bees, but they did not investigate the reason for this finding.

Very few studies assessed the value of occasional flower-visitors, such as ladybird beetles and milkweed bugs, in terms of pollen loads carried by individuals. Hawkeswood & Turner (2002) observed ladybird beetles feeding on the pollen of grass species while crawling over the inflorescence, and stated that they might play a small role in pollination, but mostly regarded them as pollen robbers, as grasses are generally wind-pollinated. Archimowitsch (1923) listed ladybird beetles as casual pollen vectors of sugar-beet in their hunt for aphid pray. Mawdsley & Sithole (2010) qualitatively assessed the pollen loads on various insect visitors to flowering savannah plant species and found that ladybird beetles carried pollen loads they described as light (pollen visible but not dense). No studies were found in the literature that evaluated pollen loads carried by milkweed bugs. This study appears to be the first to do so and showed that *S. pandurus* individuals that visited male-sterile umbels carried amounts of pollen grains that were not significantly different to those carried by honeybees also foraging on male-sterile umbels. The pollen carried by *S. pandurus* mostly adhered to bristles on the legs, with fewer grains clinging to the ventral thorax and abdomen.

Calliphorid flies are known to be efficient carriers of pollen loads for several palm and tree species (Barfod *et al.*, 2003; Griffin *et al.*, 2009; Sharma *et al.*, 2011). Here on hybrid onion flowers, calliphorid flies were also noted to carry pollen grains, but did not carry as many as honeybees or milkweed bugs, although more than ladybirds.

Overall, the findings here suggest that insect visitors recorded carrying pollen could potentially affect pollination of the hybrid onion florets they visited, because of the presence of onion pollen on their bodies. However, the pollen loads carried by *H. variegata* and *S.*

pandurus visitors on male-sterile umbels were contaminated with greater amounts of foreign pollen, which could possibly influence their efficacy as onion pollinators and suggests that onion flowers are not their primary choice of host flower. In addition, the low visitation frequency of these visitors and their slower speed of moving between blooming umbels, suggests that their role as pollinators would be minimal.

Factors that influence honeybee visitation frequency

Annual rainfall in the low-lying central valley of the Klein Karoo ranges between 100 to 300 mm, while the mountainous areas receive more than 1,000 mm annually, because of the orographic rainfall gradients and rain shadow effects that is characteristic of the Klein Karoo (Le Maitre *et al*., 2009). The southern Karoo, on the other hand, receives about 170 mm of rain annually, which is unpredictable and highly variable (Cowling, 1986; Dean & Milton, 1995). Local farmers throughout the production region reported that they experienced a water scarcity during 2010 with water supply running low (farmers pers. comm.). The lower amount of rainfall received during 2010 could have led to less abundant floral resources sustaining pollinator populations. The flower display of the Karoo vegetation is very dependent on the amount of annual rainfall (Mayer *et al*., 2006) and plants flower abundantly after good rains.

In the Klein Karoo, the majority of managed honeybee hives used for onion hybrid seed pollination are permanently residing on farm land, while managed hives are generally externally sourced for pollination purposes in the southern Karoo (refer to Table 3.6). In addition, the recent discovery of the highly infectious American Foul Brood disease in managed colonies during the first quarter of 2009, led to a decline of the relocation of managed colonies in an effort to prevent the disease from spreading (Allsopp, 2009; Steyn, 2009). Honeybee colonies resident in the Klein Karoo build up in late winter and early spring (August-September) when floral resources become abundant after winter and early spring rainfall events and warmer day temperatures, and the number of colonies will escalate, especially after good rains and abundant flowering displays (Hepburn and Guillarmod, 1991). In my model explaining honeybee visitation, annual rainfall was more important in determining honeybee visitation frequency, than managed hive stocking density, or the percentage of available natural habitat. Higher honeybee visitation frequency in the year with higher rainfall suggests that resident, managed honeybee colonies, and wild honeybee colonies, were most likely supported and boosted by more abundant floral resources after the

good rain season and therefore the higher numbers of honeybees seen in this year were related to colony size and strength.

Honeybee visitation frequency on onion has been reported to be proportional to colony population size (Waller *et al.*, 1985). The supply of large and healthy honeybee colonies is therefore important to ensure the availability of a sufficient number of worker bees to pollinate the onion hybrid seed crops. My results suggest that the size of resident managed, as well as wild honeybee populations would significantly determine the abundance of honeybee foragers on blooming onion hybrid seed crops.

Another important factor that may influence honeybee visitation is the time the region receives rain. Flowering times are largely determined by the timing of rainfall events. The rainfall season varies from west to east throughout the Klein Karoo, whereby the western region (Montagu) receives winter rainfall which progressively shifts to summer rainfall events received by the east (Willowmore) (Le Maitre *et al.*, 2009), while rainfall in the southern Karoo is highly sporadic (Cowling, 1986; Dean & Milton, 1995). Wild plants in flower may attract honeybee foragers away from blooming onion hybrid seed crops, especially so when the flowering times of the natural veldt coincide with the flowering of the hybrid onion crops. Early rainfall events (during July, August and early September) lead to the veldt flowering early so that by the time the hybrid onion crops come into flower in late October to early November, the natural veldt does not offer sufficient floral resources to attract insect foragers away from the blooming onion hybrid seed crops. However, if rain falls later in the season (late September to October), the natural vegetation will flower during the hybrid onion blooming period and possibly attract honeybees away from the less desirable hybrid onion crops (Waller, 1970; Nye *et al.*, 1973). Consequently, hybrid onion crops with more natural habitat may compete with flowering wild plants for honeybee visitation if the natural veldt flowers during late spring.

Low rainfall events may cause additional secondary factors that may also have negative effects on honeybee visitation to crops. Although it is unlikely that low rainfall would cause water stress in onion plants, since onion crops are irrigated according to very specific irrigation schedules (F. van der Merwe, pers. comm.), the water sourced from large dams that is used to irrigate the crops may be of lower quality because of evaporation, causing higher salt concentrations, which may deter honeybees from foraging on the onion flowers when sprinkle irrigation is used, or when this water causes increased soil-salinity (P. Burger, pers.

comm.). Potassium concentrations in the nectar of onion flowers have been linked with soil-salinity and these higher potassium concentrations appear to influence the attractiveness of the nectar for honeybees (Waller *et al.*, 1972). Further research is needed to test the effects of secondary factors, caused by the variability in rainfall events, on honeybee visitation frequency.

CONCLUSION

Only a fraction of the abundance and richness of anthophile insects that were sampled within onion hybrid seed crops in chapter 2 actually visited the blooming umbels. The honeybee was by far the most frequent visitor and foraged predominantly for nectar. The population of foraging honeybees was not equally spread among male-fertile and male-sterile umbels, with more honeybees foraging on male-fertile lines. Honeybees carried substantial amounts of onion pollen on their bodies which confirmed them as important onion pollen vectors. On the other hand, the low numbers and poor visitation rates of non-*Apis* visitors combined with the lower quantities of onion pollen carried, would suggest that they offer pollination services to a much lesser extent in comparison to honeybees, even though they actively foraged on onion florets for nectar. The source of the honeybee foragers could not be positively established, because honeybee visitation frequency was not significantly related to managed hive density, nor to percentage natural habitat. Nevertheless, annual rainfall explained honeybee visitation frequency on the onion flowers, and this appears to be particularly important for the Klein Karoo region where farmers rely on resident, managed and wild honeybee colonies. Abundant wild flower resources after good annual rainfall would boost and support larger pollinator populations, which might also explain the positive correlation between honeybee visitation frequency and anthophile diversity on onion umbels. However, the timing of rainfall events might be crucial and would determine whether hybrid onion crops would have to compete with wild flower resources for pollinator visitation. The effect of secondary factors caused by annual rainfall variation on insect visitation to onion flowers should be investigated further.

REFERENCES

Allsopp, M.H., 2009. *Bee disease threatens South African apiculture*. Plant Protection News, N79, Agricultural Research Council: Plant Protection Research Institute.

Archimowitsch, A., 1923. Control of pollination in sugar-beet. *The Botanical Review* 15, 613–628.

Armstrong, J.A., 1979. Biotic pollination mechanisms in the Australian flora — a review. *New Zealand Journal of Botany* 17, 467–508.

Barfod, A.S., Burholt, T., Borchsenius, F., 2003. Contrasting pollination modes in three species of *Licuala* (Arecaceae: Coryphoideae). *Telopea* 207–224.

Bates, D., Maechler, M., Bolker, B., 2011. lme4: Linear mixed-effects models using S4 classes.

Bertolaccini, I., Nunez-Perez, E., Tizado, E.J., 2008. Effect of wild flowers on oviposition of *Hippodamia variegata* (Coleoptera: Coccinellidae) in the laboratory. *Journal of Economic Entomology* 101, 1792–1797.

Bohart, G.E., Nye, W.P., Hawthorn, L.R., 1970. *Onion pollination as affected by different levels of pollinator activity*. Utah Agricultural Experiment Station Bulletin 482.

Brewster, J.L., 2008. *Onions and other vegetable Alliums, 2nd Edition*. CAB International, Oxon.

Caron, D.M., Lederhouse, R.C., Morse, R.A., 1975. Insect pollinators of onion in New York State. *Hortscience* 10, 273–274.

Chandel, R.S., Thakur, R.K., Bhardwaj, N.R., Pathania, N., 2004. Onion seed crop pollination: a missing dimension in mountain horticulture. *Acta Horticulturae* 631, 79–86.

Cowling, R.M., 1986. *A description of the Karoo Biome Project*. South African National Scientific Programmes Report No. 122. Pretoria.

Currah, L., Ockendon, D.J., 1983. Onion pollination by blowflies and honeybees in large cages. *Annals of Applied Biology* 103, 497–506.

Currah, L., Ockendon, D.J., 1984. Pollination activity by blowflies and honeybees on onions in breeders' cages. *Annals of Applied Biology* 105, 167–176.

Dafni, A., Kevan, P.G., Husband, B.C. (Eds.), 2005. *Practical pollination biology*. Enviroquest, Ontario.

Dean, W.R.J., Milton, S.J., 1995. Plant and invertebrate assemblages on old fields in the arid southern Karoo, South Africa. *African Journal of Ecology* 33, 1–13.

Dowker, B.D., Currah, L., Horobin, J.F., Jackson, J.C., Faulkner, G.J., 1985. Seed production of an F_1 hybrid onion in polyethylene tunnels. *Journal of Horticultural Science* 60, 251–256.

Free, J.B., 1993. *Insect pollination of crops*. Academic Press, London, UK.

Free, J.B., Williams, I.H., 1972. The transport of pollen on the body hairs of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus spp*. L.). *Journal of Applied Ecology* 9, 609–615.

George, R.A.T., 1999. *Vegetable seed production, 2nd Edition*. CAB Interantional Publishing, New York.

Griffin, A.R., Hingston, A.B., Ohmart, C.P., 2009. Pollinators of *Eucalyptus regnans* (Myrtaceae), the world's tallest flowering plant species. *Australian Journal of Botany* 57, 18–25.

Hawkeswood, T.J., Turner, J.R., 2002. Observations on the adults of *Micraspis frenata* (Erichson, 1842) (Coleoptera: Coccinellidae) feeding on the pollen of native and non-native grasses (Poaceae) in eastern New South Wales, Australia. *Journal of the Entomological Research Society* 4, 21–29.

Hepburn, H.R., Guillarmod, J., 1991. The Cape honeybee and the fynbos biome. *South African Journal of Science 87,* 70–73.

Hilton-Tailor, C., Le Roux, A., 1989. Conservation status of the fynbos and karoo biomes, in: Huntley, B.J. (Ed.), *Biotic diversity in southern Africa*. Oxford University Press, Cape Town, pp. 202–223.

Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeileis, A., 2008. Implementing a class of permutation tests: the coin package. *Journal of Statistical Software* 28, 1–23.

Howlett, B.G., Donovan, B.J., McCallum, J.A., Newstrom, L.E., Teulon, D.A.J., 2005. Between and within field variability of New Zealand indigenous flower visitors to onions. *New Zealand Plant Protection* 58, 213–218.

Jones, H.A., Clarke, A.E., 1943. Inheritance of male sterility in the onion and the production of hybrid seed. *Proceedings of the American Society for Horticultural Science* 43, 189–194.

Kendall, D.A., Solomon, M.E., 1970. *Insect pollination of fruit trees*. Report of the Agriculture Horticultural Research Station, University of Bristol.

Kendall, D.A., Solomon, M.E., 1973. Quantities of pollen on the bodies of insects visiting apple blossom. *Journal of Applied Ecology* 10, 627–644.

Le Maitre, D.C., Colvin, C., Maherry, A., 2009. Water resources in the Klein Karoo: the challenge of sustainable development in a water-scarce area. *South African Journal Of Science* 105, 39–48.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1968. Onion pollination in New York. *New York's Food and Life Sciences Bulletin* 1, 8–9.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1972. Distribution and behavior of honey bees on onion. *Environmental Entomology* 1, 127–129.

Lundgren, J.G., 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. *Biological Control* 51, 294–305.

Mann, L.P., Woodbury, G.W., 1969. The effect of flower age, time of day and variety on pollen germination of onion, *Allium cepa* L. *Journal of the American Society of Horticultural Science* 94, 102–104.

Mawdsley, J.R., Sithole, H., 2010. Diversity and abundance of insect visitors to flowers of trees and shrubs in a South African savannah. *African Journal of Ecology* 48, 691–698.

Mayer, C., Soka, G., Picker, M.D., 2006. The importance of monkey beetle (Scarabaeidae: Hopliini) pollination for Aizoaceae and Asteraceae in grazed and ungrazed areas at Paulshoek, Succulent Karoo, South Africa. *Journal of Insect Conservation* 10, 323–333.

McGregor, S.E., 1976. Common vegetables for seed and fruit, in: McGregor, S.E. (Ed.), *Insect Pollination of Cultivated Crop Plants*. United States Department of Agriculture, Washington.

Moffett, J.O., 1965. Pollinating experimental onion varieties. *American Bee Journal* 105, 378.

Moll, R.H., 1954. Receptivity of the individual onion flower and some factors affecting its duration. *Proceedings of the American Society for Horticultural Science* 64, 399–404.

Muller, H., 1883. *The fertilisation of flowers*. Macmillan, London.

Munawar, M.S., Raja, S., Niaz, S., Sarwar, G., 2011. Comparative performance of honeybees (*Apis mellifera* L.) and blow flies (*Phormia terronovae*) in onion (*Allium cepa* L.) seed setting. *Journal of Agricultural Research* 49, 49–56.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., De Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.

Nye, W.P., Shasha'a, N.S., Campbell, W.F., Hamson, A.R., 1973. Insect pollination and seed set of onions (*Allium cepa* L.). *Utah Agricultural Experiment Station Research Report* No. 6, 15.

Nye, W.P., Waller, G.D., Waters, N., 1971. Factors affecting pollination of onions in Idaho during 1969. *Journal of the American Society of Horticultural Science* 96, 330–332.

Parker, F.D., 1982. Efficiency of bees in pollinating onion flowers. *Journal of the Kansas Entomological Society* 55, 171–176.

Parker, F.D., Hatley, C.L., 1979. Onion pollination: viability of onion pollen and pollen diversity on insect body hairs., in: *Proceedings of the Fourth International Symposium on Pollination*. Maryland Agricultural Experiment Station Special Miscellaneous Publication 1, pp. 201–206.

R Development Core Team, 2011. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. (www.r-project.org)

Roubik, D.W., 2001. Ups and downs in pollinator populations: when is there a decline? *Conservation Ecology* 5, 2 (online at: http://www.consecol.org/vol5/iss1/art2/).

Saeed, S., Sajjad, A., Kwon, O., Kwon, Y.J., 2008. Fidelity of Hymenoptera and Diptera pollinators in onion (*Allium cepa* L.) pollination. *Entomological Research* 38, 276–280.

Sajjad, A., Saeed, S., Masood, A., 2008. Pollinator community of onion (*Allium cepa* L.) and its role in crop reproductive success. *Pakistan Journal of Zoology* 40, 451–456.

Schittenhelm, S., Gladis, T., Rao, V.R., 1997. Efficiency of various insects in germplasm regeneration of carrot, onion and turnip rape accessions. *Plant Breeding* 116, 369–375.

Shanmugasundaram, S., 1998. *Onion cultivation and seed production*. Shanhua, Tainan, AVRDC.

Sharma, M. V, Uma Shaanker, R., Leather, S.R., Vasudeva, R., Shivanna, K.R., 2011. Floral resources, pollinators and fruiting in a threatened tropical deciduous tree. *Journal of Plant Ecology* 4, 259–267.

Steyn, T., 2009. Bysiekte in tot 85% van korwe. *Die Burger, 14 July,* p.6.

Thorp, R.W., 1979. Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. *Annals of the Missouri Botanical Garden* 66, 788–812.

Voss, R.E., Murray, M., Bradford, K., Mayberry, K.S., Miller, I., 1999. *Onion seed production in California*. University of California, Devision of Agriculture and Natural Resources.

Waller, G.D., 1970. Problems with onion pollination in Arizona. *Arkansas Agricultural Extension Service Miscellaneous Publications* 127, 145–149.

Waller, G.D., Carpenter, E.W., Ziehl, O.A., 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. *Journal of the American Society of Horticultural Science* 97, 535–539.

Waller, G.D., Gary, N.E., Chester Jr, S.T., Karim, M.S., Martin, J.H., Vaissière, B.E., 1985. Honeybee colony populations and foraging rates on onion seed-fields in Arizona. *Journal of Apicultural Research* 24, 93–101.

Walsh, R.S., 1965. Pollination of onion plants by honeybees. *New Zealand Beekeeper* 27, 18–20.

Williams, I.H., Free, J.B., 1974. The pollination of onion (*Allium cepa* L.) to produce hybrid seed. *Journal of Applied Ecology* 11, 409–417.

Williams, N.M., Minckley, R.L., Silveira, F.A., 2001. Variation in native bee faunas and its implications for detecting community changes. *Conservation Ecology* 5, 7. (online at: http://www.consecol.org/vol5/iss1/art7/)

Witter, S., Blochtein, B., 2003. Effect of pollination by bees and other insects on the production of onion seeds. *Pesquisa Agropecuária Brasileira* 38, 1399–1407.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. *Mixed effects models and extensions in ecology with R*. Springer, New York.

Table 3.1: Visitation frequency (mean ± S.D.) of insect visitor guilds on the two parental lines (time of day combined) during a total of 2880 observations on hybrid onion umbels.

	Parental line	
Visitor group	MF	MS
A.m. capensis	6.29 ± 6.28^{a}	4.54 ± 4.76^{b}
Coccinellidae	0.24 ± 0.63^{a}	0.29 ± 0.59^{a}
Diptera	0.15 ± 0.48^{a}	0.17 ± 0.42^{a}
Hemiptera	0.05 ± 0.24^{a}	0.01 ± 0.11^{b}
non-*Apis* bees	0.02 ± 0.15^{a}	0.02 ± 0.14^{a}
Wasps	0.01 ± 0.14	0.02 ± 0.14
Butterflies	0.00 ± 0.06	0.01 ± 0.11
Coleoptera (other)	0.00 ± 0.06	0.00 ± 0.00

MF = male-fertile umbels, MS = male-sterile umbels. Different letters in superscript denote significant differences between parental lines. (Approximative Wilcoxon Mann-Whitney rank-sum tests, $P < 0.05$)

Table 3.2: Observations of flower-visitor behaviour in terms of resource collection, contact with floral reproductive parts, and number of florets visited by each visitor. Data are shown for the four most abundant insect visitor groups recorded on hybrid onion umbels.

		Resource collection				Reproductive contact			
Visitor guild	Parent	Nectar	Pollen	Both	N	Anther	Stigma	Both	N
A.m. capensis	MF	71.7%	2.6%	25.5%	572	30.8%	0.3%	68.3%	603
	MS	89.4%			464	14.2%	1.4%	83.6%	487
Coccinellidae	MF	94.8%	0%	2.1%	96	49.0%	9.2%	31.6%	98
	MS	93.4%			106	19.6%	9.3%	66.4%	107
Diptera	MF	97.8%	0%	0%	46	26.9%	9.6%	59.6%	52
	MS	93.0%			57	21.8%	12.7%	65.5%	55
Hemiptera	MF	100%	0%	0%	12	0%	8.3%	91.7%	12
	MS	100%			3	0%	33.3%	66.7%	3

		# of Florets visited		
		<10	>10	N
A.m. capensis	MS	60.0%	40.0%	867
Coccinellidae	MS	51.0%	49.0%	153
Diptera	MS	58.1%	41.9%	210
Hemiptera	MS	16.7%	83.3%	18

MF = male-fertile umbels, MS = male-sterile umbels.

Table 3.3: Pollen grain counts (mean ± S.D.) and estimated percentages of onion pollen on the bodies of frequent, hybrid onion flower-visitors.

Visitor	Parent	N (individuals)	Pollen grains	N (samples)	Total onion	Total other	% Onion pollen
A.m. capensis	MF	31	109,613 ± 70,370[a]	5	3562	45	98.8%
	MS	46	30,998 ± 31,846[b]	5	860	32	96.4%
Calliphoridae	MF	16	7,130 ± 8,530[a]	5	994	21	97.9%
	MS	15	5,250 ± 7,670[a]	5	307	18	94.5%
H. variegata	MF	17	2,729 ± 2,049[a]	5	118	14	89.4%
	MS	21	866 ± 704.37[b]	5	65	38	63.1%
S. pandurus	MF	13	21,407 ± 21,776[a]	1	101	14	87.8%
	MS	8	13,195 ± 10,103[a]	1	73	32	69.5%

Different letters in superscript indicate significant differences in pollen loads on insect visitors sampled from male-fertile (MF) and male-sterile (MS) umbels. (Approximative Wilcoxon Mann-Whitney rank-sum tests, $P < 0.05$)

Table 3.4: Spearman correlation coefficients between the continuous explanatory variables used in the generalized linear mixed effects model as fixed terms. A cut-off correlation of 0.70 was used as selection criteria and those correlations rounding to or above this value is indicated in bold.

Fixed variables	Natural habitat (%)	Managed hive density	Temperature	Wind speed	Annual rainfall
Managed hive density	0.12				
Temperature (˚C)	-0.15	-0.13			
Wind speed (km/h)	-0.29	-0.13	-0.15		
Annual rainfall (mm)	**-0.66**	-0.12	0.12	0.39	
Field size	-0.25	-0.34	0.01	0.23	0.09

Table 3.5: Results of the (GLMM) analyses of the visitation frequency of honeybees to hybrid onion umbels. The Z-values from likelihood ratio tests and the direction of the relationships are shown for the variables in the two final models. Blank spaces represent dropped variables following stepwise deletion. The number of observations was 520 and there were 13 farms. (Random factor SD: M1 = 0.54, M2 = 0.53)

Model	AIC	Natural habitat (%)	Hive density (/ha)	Temperature (˚C)	Wind speed (km/h)	Total annual rainfall (mm)	Field size (ha)	Method of irrigation
M1	1073					6.41*** (+)		
M2	1075		-0.518 (-)			6.47*** (+)		

(***$P < 0.001$, **$P < 0.01$, *$P < 0.05$)

Table 3.6: Managed hive stocking densities of the 13 onion hybrid seed farms used for data analysis with indications of whether the hives were resident or imported from outside the production region. (refer to Appendix A for farm details)

Farm	Managed hive density	Production region	Residential/Imported
R	2.00	southern Karoo	Imported
C	4.74	Klein Karoo	Residential
J	5.59	Klein Karoo	Residential
I	6.26	Klein Karoo	Residential
G	7.20	Klein Karoo	Residential
F	7.50	Klein Karoo	Residential
L	7.94	southern Karoo	Imported
M	8.50	southern Karoo	Imported
P	8.56	southern Karoo	Imported
D	8.70	Klein Karoo	Residential
K	8.91	southern Karoo	Imported
Q	10.00	southern Karoo	Imported
E	10.43	southern Karoo	Imported

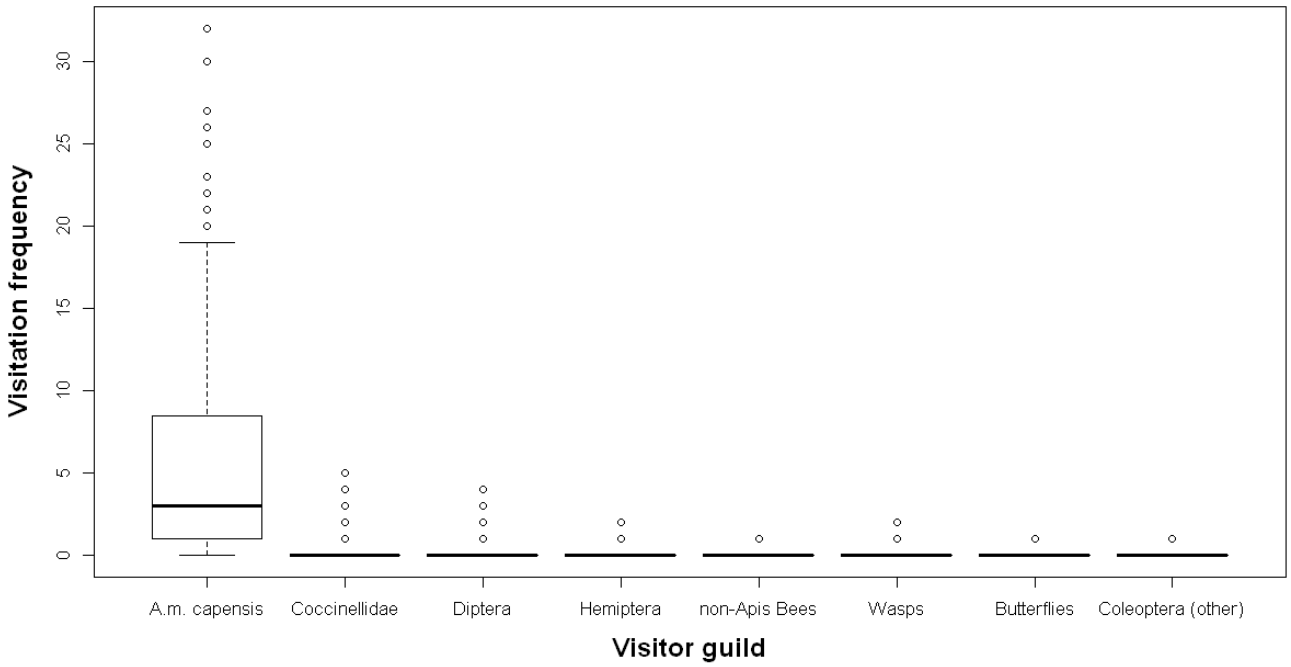


Figure 3.1: Visitation frequency for each insect guild observed on 4 umbels in 4 minutes on MF and MS hybrid onion umbels (median with lower and upper quartile and non-outlier range, open circles indicate outliers).

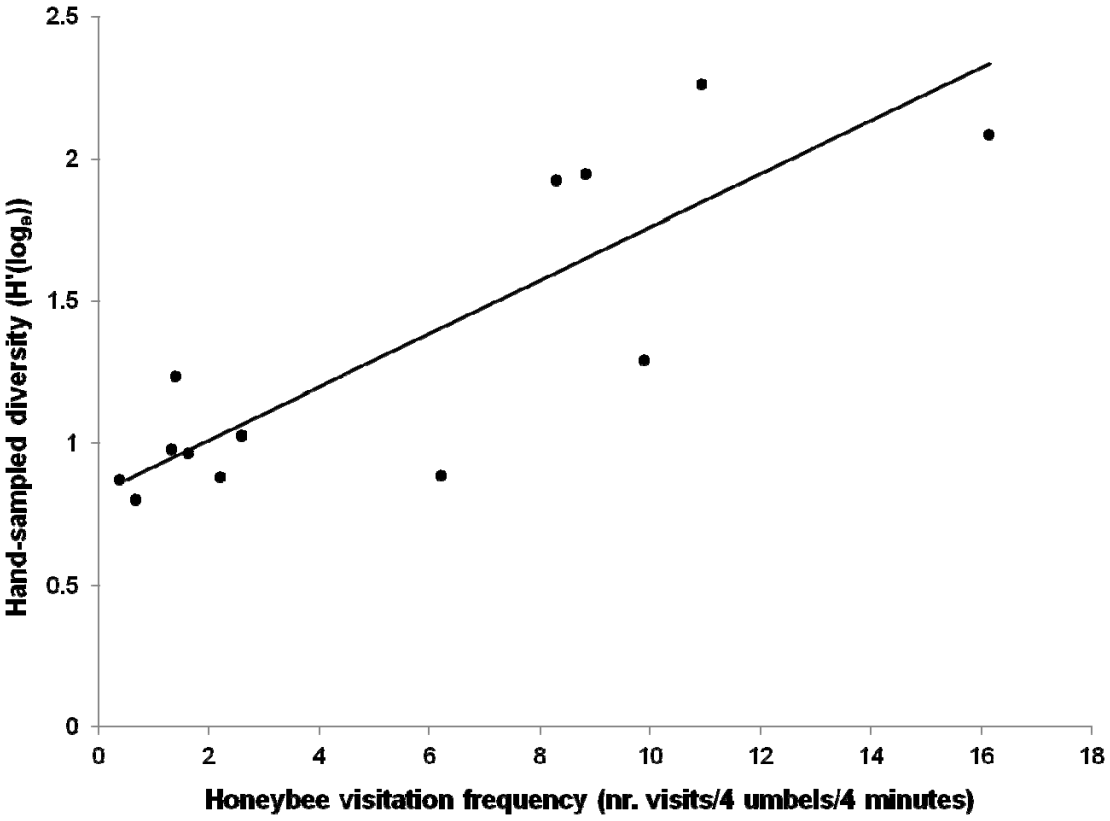


Figure 3.2: The relationship between observed honeybee visitation frequency and the diversity of hand-sampled insects that foraged on hybrid onion umbels of 13 farms.

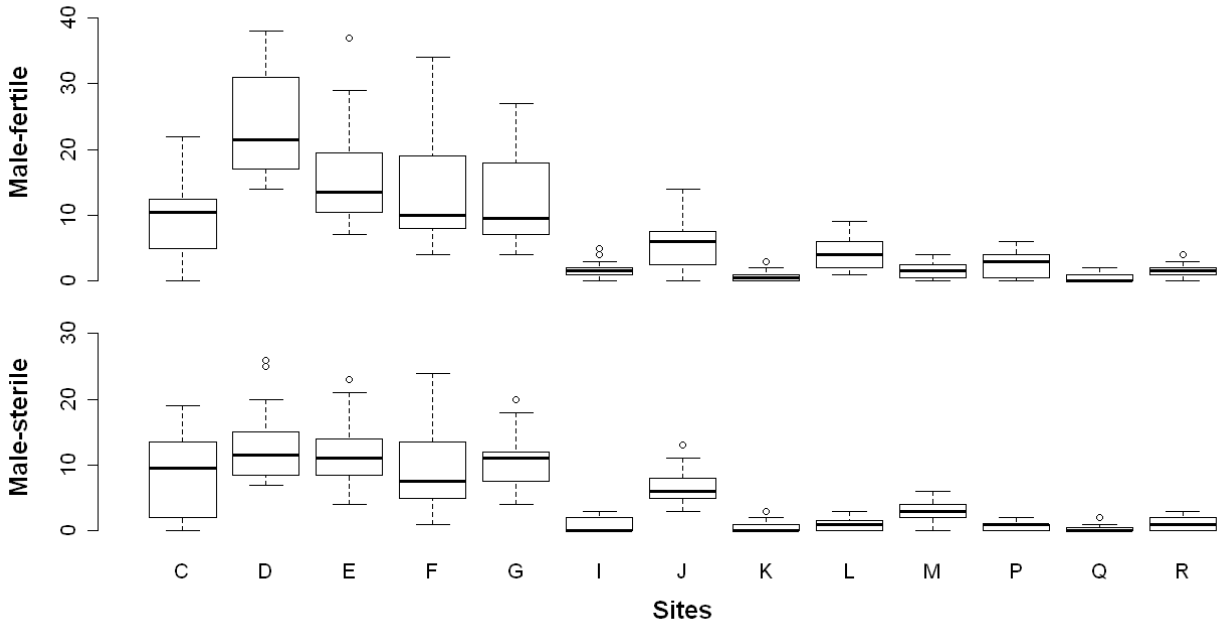


Figure 3.3: Visitation frequency of *A.m. capensis* individuals observed on 4 umbels in 4 minutes on male-fertile and male-sterile umbels at each site. Sites C-G were sampled in 2009, and sites I-R were sampled in 2010 (median with lower and upper quartile and non-outlier range, open circles indicate outliers).

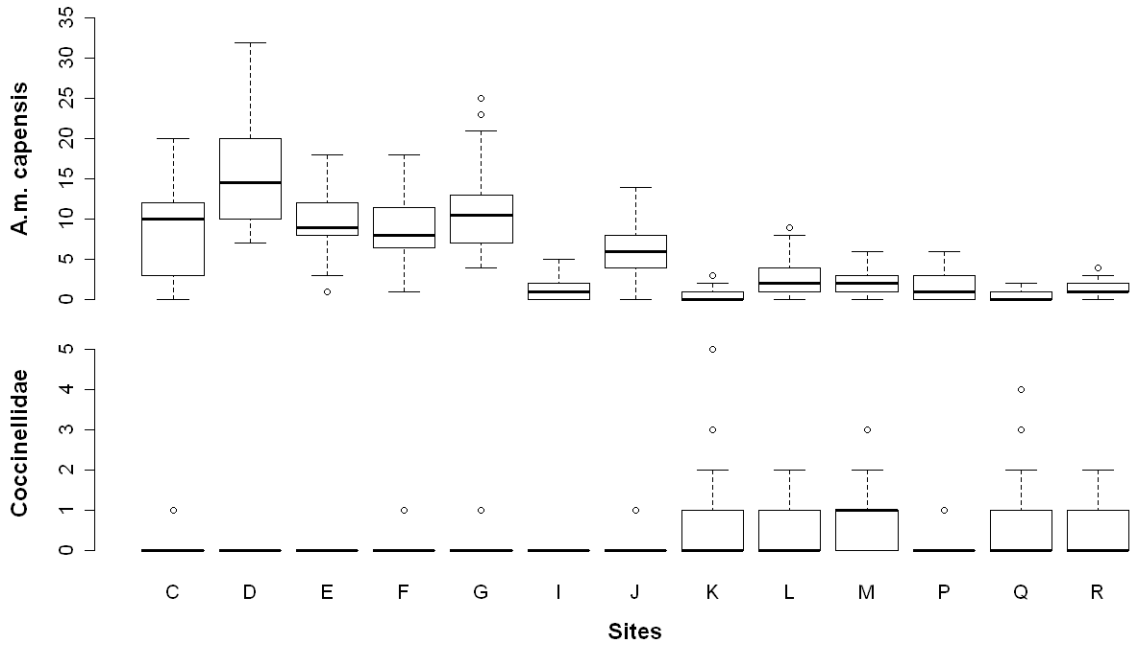


Figure 3.4: Visitation frequency for honeybees and most abundant non-*Apis* visitor, ladybirds, the two most abundant insect visitor guilds observed on 4 umbels in 4 minutes on male-fertile and male-sterile hybrid onion umbels at each site (median with lower and upper quartile and non-outlier range, open circles indicate outliers).

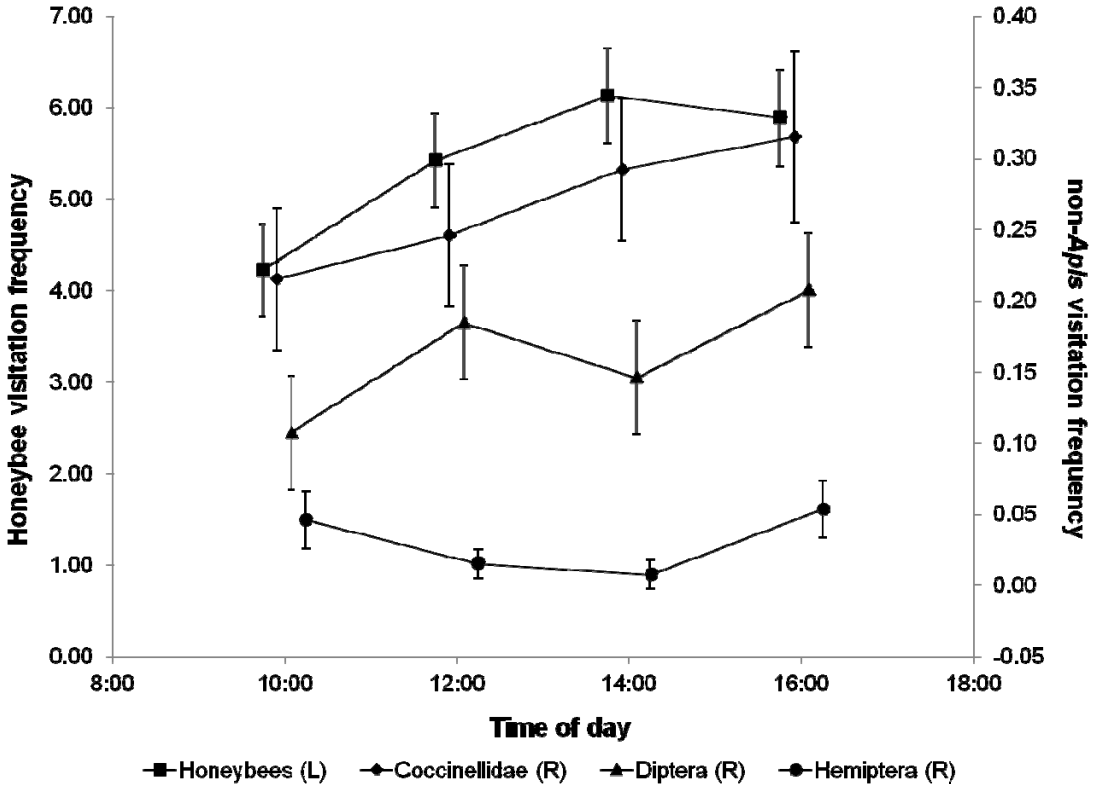


Figure 3.5: Visitation frequency (mean ± S.E.) of honeybees and the three most abundant non-*Apis* insect flower-visitors recorded per minute on four umbels at each time interval over a daily period.

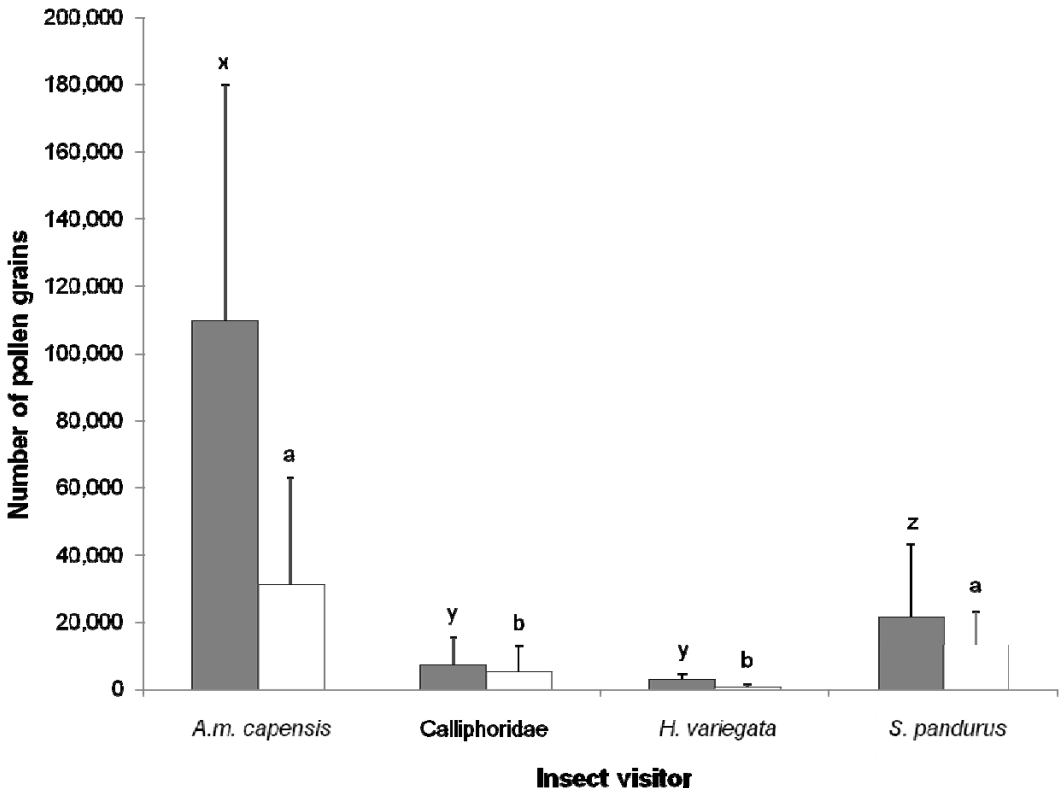


Figure 3.6: Pollen load counts (mean ± S.D.) on the bodies of honeybees and the three most abundant non-*Apis* insect visitors sampled from male-fertile (grey bars) and male-sterile (white bars) hybrid onion umbels. Columns with the same letters indicate that there is no significant differences in pollen loads between insect visitors sampled on male-fertile (x-z) or male-sterile (a,b) umbels.

4

ONION HYBRID SEED PRODUCTION IN SOUTH AFRICA: DOES IT BENEFIT FROM POLLINATION ECOSYSTEM SERVICES?

ABSTRACT

Onion hybrid seed production is totally dependent on insect pollination for successful yields. Even though a diversity of insects visit onion flowers, honeybees are typically the main pollinators of onion seed crops. The Cape honeybee is indigenous and essentially endemic to the Western Cape, South Africa where onion hybrid seed are produced, and is also used to populate managed hives. I aimed to assess whether onion hybrid seed farmers benefit from pollination ecosystem services from wild honeybees and from the presence of a diversity of flower-visiting insects within the crops. Results showed that honeybee visitation significantly increased seed yield, but seed yield was not influenced by general anthophile diversity or non-*Apis* visitation. However, it remains unclear whether improved seed yield was due to the stocking rates of managed honeybee colonies, or to the percentage of natural habitat near the crops as a source of wild honeybees. Other factors not investigated here were possibly more influential in determining honeybee visitation rates on the onion flowers; for example, varietal attractiveness, competition from wild flowers or climatic conditions, and that the entire pollinator population possibly responded similarly to these factors. The ratio of male-fertile to male-sterile planting rows seemed to affect seed yield and should be considered as an important factor in the pollination of onion hybrid seed.

INTRODUCTION

Insect pollination is essential for the production of 87 (70%) of the 124 food crops grown for direct human consumption worldwide (Klein *et al.*, 2007), while the total economic value of crop pollination was an estimated €153 billion in 2009 (Gallai *et al.*, 2009). Managed honeybees (*Apis mellifera* L.) have long been considered the most important pollinating agents of insect pollinated crops (Free, 1993; Southwick and Southwick, 1992). However, during recent years, and in response to a threatening global decline of pollinators (Buckmann and Nabhan, 1996; Steffan-Dewenter *et al.*, 2005; Potts *et al.*, 2010), focus has shifted to the value and potential of pollination ecosystem services received from unmanaged, mostly non-

Apis insect pollinators (Aizen et al., 2009; Allsopp et al., 2008; Breeze et al., 2011; Carvalheiro et al., 2010; Klein et al., 2007; Rader et al., 2012; Westerkamp and Gottsberger, 2002, 2001, 2000; Winfree et al., 2007). For some crops, it has been found that native bees are more effective pollinators than honeybees (Cane, 1997; Greenleaf and Kremen, 2006a; Klein et al., 2003; Westerkamp and Gottsberger, 2001, 2000).

A substantial amount of work has been done on pollination ecosystem services received by various agricultural crops, and has often been linked to the availability of natural habitat near the crops (see for example Klein *et al*. (2012) for almond; Heard and Exley (1994) and Blanche *et al*. (2006) for macadamia; Kremen *et al*. (2002, 2004) for watermelon; Chacoff and Aizen (2006) for grapefruit; Carvalheiro *et al*. (2010) for mango; Hoehn *et al*. (2008) for pumpkin; and Greenleaf and Kremen (2006) for tomato). Greenleaf and Kremen (2006b) found that wild bees foraging on hybrid sunflower delivered a significant indirect pollination ecosystem service in that inter-specific interactions between wild bees and honeybees increased the pollination efficiency of honeybees 5-fold, which was more important than direct pollination from wild bees. Similar results were reported by Degrandi-Hoffman and Watkins (2000) for hybrid sunflower.

A great deal of work has been done on the pollination of onion hybrid seed (*Allium cepa* L.), primarily because problems with pollination are often experienced since onion flowers are generally unattractive to honeybees (Waller *et al*., 1972). Onion hybrid seed crops are often visited by a diversity of anthophile insects (Bohart et al., 1970; Caron et al., 1975; Howlett et al., 2005; Lederhouse et al., 1968; Mayer and Lunden, 2001; Saeed et al., 2008; Sajjad et al., 2008; Walker et al., 2011; Williams and Free, 1974; Witter and Blochtein, 2003), and some insects have been found to be more effective than others in pollinating onion flowers (Currah and Ockendon, 1984, 1983; Dowker et al., 1985; Moffett, 1965; Parker, 1982; Saeed et al., 2008; Sajjad et al., 2008; Schittenhelm et al., 1997; Waller et al., 1985; Walsh, 1965; Wilkaniec et al., 2004; Williams and Free, 1974; Witter and Blochtein, 2003). However, the degree to which onion hybrid seed crops benefit from pollination ecosystem services delivered by wild pollinators as opposed to pollination services delivered by managed pollinators is still unclear.

Seed production is valued as an important contributor to the economy of the Klein Karoo (Van der Walt, 1999). The South African onion seed market has been estimated to be worth USD 2.7 million in 2000 (Van der Walt, 2002). Managed honeybee colonies are the principle

means used to pollinate hybrid onion crops in South Africa, but honeybees, in this case *Apis mellifera capensis* Esch., is indigenous to the Western Cape (Hepburn and Radloff, 1998) and is inherently part of the wild pollinator community, thus also able to deliver pollination ecosystem services without cost to the farmers. The recommended stocking density of managed honeybee colonies for hybrid onions are about 10 hives/ha (Johannsmeier, 2001), but some South African farmers rely on wild honeybee colonies to various degrees to pollinate the crops.

Onion varieties are known to differ in their attractiveness to honeybee foragers (Hagler and Waller, 1991), which can have significant consequences for seed yield. Also, considerable variability in seed production has been noted between onion cultivars, with Jones (1923) giving estimates of 336 kg/ha for red varieties, 448 kg/ha for yellow and brown varieties and 223 kg/ha for white varieties. Thus, varietal issues in terms of attractiveness to honeybees and cultivars' seed production capabilities need to be considered when assessing seed yields associated with possible pollination deficits across a number of hybrid onion varieties.

Other factors which could affect the seed yield of onions include the synchrony in blooming dates of parental lines (Pathak, 2000), weather conditions during flowering (Woyke, 1981), mother bulb size (Levy *et al.*, 1981), the number of florets per umbel (Erickson and Gabelman, 1954), stigma receptivity (Moll, 1953), and pollen viability (Mann and Woodbury, 1969). In addition, several crop management practices are known to affect onion pollination and seed yield. These include irrigation techniques (Brown *et al.*, 1951; Levy *et al.*, 1981), use of nitrogen fertilizers (Levy *et al.*, 1981; Stuart and Griffin, 1946), use of insecticides (Long and Morandin, 2011), ratios of planted male-fertile to male-sterile rows (Franklin, 1958), and the distance between planted male-fertile and male-sterile rows (Erickson and Gableman, 1956).

The onion is self-compatible but self-pollination is prevented when producing hybrid seed through the breeding of F1 hybrid parental lines, where male-sterile plants that do not produce pollen, are cross-pollinated with male-fertile plants producing pollen (Williams and Free, 1974). Vaissière *et al.* (2001) reported that airborne pollen contributed approximately 11% to pollination effectiveness of open-pollinated hybrid onion flowers. It is therefore clear that the demand for pollination services is greater where hybrid seed is produced (Southwick and Southwick, 1992). Carlson (1974) reported that onion varieties had greater seed yields where honeybee visitation frequency was higher, highlighting the importance of honeybees to hybrid onion pollination and seed yield. For South African systems, the honeybee (managed

and wild) seems to be an important pollinator of hybrid onion because of sufficient visitation rates and considerable onion pollen loads carried by individual honeybees foraging on the umbels as reported in chapter 3.

Aims

The main aim of this chapter was to determine whether onion hybrid seed production in South Africa benefits from pollination ecosystem services delivered by unmanaged insect visitors, by assessing the degree to which seed yield is related to one or more of the following factors: natural habitat availability; anthophile diversity; managed hive density; and honeybee and non-*Apis* visitation frequency. Simultaneously, two management practices were also investigated for their impact on seed yield; the ratio of male-fertile to male-sterile parent rows planted within the crops and the methods of irrigation used. Assessing these variables will hopefully offer insights into the degrees of importance of managed and wild pollinators in contributing to onion hybrid seed yield, and highlight possible pollination ecosystem service benefits farmers gain from wild pollinator communities.

METHODS

Study sites

Data was collected from 13 healthy onion hybrid seed crops growing 12 different cultivars (five farms in 2009 (C-G) and eight farms in 2010 (I-M, P-R)) grown in the Klein Karoo and southern Karoo regions in the Western Cape (refer to Table A.1, and Figure A.1 in Appendix A). The climatic characteristics of these areas are optimal for onion seed production which requires low humidity and mild cool temperatures during the initial growth phase, followed by increased temperatures later on to induce flowering (Shanmugasundaram, 1998). The main vegetation types in the low-lying areas of both regions are succulent Karoo which is characterized by open to sparse dwarf (up to 1 m tall) shrublands dominated by stem and leaf succulents and some fine-leaved evergreen shrubs (Hilton-Tailor and Le Roux, 1989). The Succulent Karoo biome is recognised as a global biodiversity hotspot with high plant diversity (Myers *et al.*, 2000). Varietal differences between the farms was based solely on coded information given by the participating seed companies, because of the sensitive nature of hybrid onion breeding information (see Table 2.1).

Landscape context

Each crop field was demarcated spatially with a GPS (GPSmap 76, Garmin). The surrounding land cover within a radius of 500m from the site edges was classified as natural or cultivated using ArcGIS 9.2 (ESRI, 2006). The National Land Cover (NLC) layer with 1 minute resolution (bgis.sanbi.org/landcover/project.asp) was used to classify the land cover surrounding the sites. From this, the percentage natural habitat surrounding each site within a 500m radius was calculated and used as a predictor variable in data analyses. The irrigation methods used were also noted for each crop and were scored as drip (D), sprinkle (S) or flood (F) irrigation.

Managed hive stocking densities

Numbers of managed hives placed within a 1 km radius from the experimental field sites at the time of flowering were counted. Ribbands (1951) reported that hived honeybee colonies had substantial losses in weight at foraging distances further than 1 km. Furthermore, Gary *et al.*, (1972) reported that the average foraging range of honeybees from distant apiaries to onion crops was 557 m because of the relative unattractiveness of onion nectar to the bees. Hives were regarded as managed if honey was harvested from it, i.e. hives were maintained and serviced (resident hives), or rented during the onion flowering period. Managed hive stocking densities were then calculated for each site by dividing the number of hives by the total hectares of blooming onion hybrid seed crop at each experimental site. No other cultivated crops bloomed at the same time as the hybrid onions which could cause possible competition for available pollinators.

Anthophile diversity

Pan trap sample data collected within the crop field sites was used to calculate average taxonomic distinctness (Δ^*) as a measure of anthophile diversity. Taxonomic distinctness (Δ) is a biodiversity measure that is less sensitive to sampling effort because it is based largely on relatedness of species within a sample (Clarke and Warwick, 1998). A branch length of 1 was used in the calculations as weight between each taxonomic level. Five groups with six different coloured traps per group (red, orange, pink, white, fluorescent yellow and blue) were positioned randomly in each field, and were placed on podiums to raise them to roughly flower level (about 1m). Traps within a group were spaced about 1.5 m apart. The traps were set up before 9h00, three quarters filled with weakly diluted soapy water (Kearns & Inouye, 1993), and left out for two days in 2009 and one day in 2010. Trapped insects were

placed in 70% ethanol for later assessment and identification. All insects caught in the six pan traps within a single group of pan traps were pooled and treated as a single sample. All insect specimens were identified to morpho-species (a list of the identified taxa appears in Appendix B). Specimens with no obvious pollinator value, like spiders and caterpillars were discarded. Reference collections of morpho-species were sent to various expert taxonomists for identification and were deposited in museum collections (see Appendix B).

*Honeybee and non-*Apis *visitation frequencies*

The average honeybee and non-*Apis* visitation frequency was calculated by recording the number of visitors to four hybrid onion umbels in four minute time windows, both on male-fertile and male-sterile rows. Four neighbouring umbels in at least 50% bloom were selected and observed while the number and identity of visitors (visitation frequency) were recorded. Visitors were later grouped into honeybee and non-Apis visitors. Honeybees from managed colonies and wild hives could not be distinguished but managed hive density within a 1,000 m radius and percentage of natural vegetation within a 500 m radius around the crops was taken as respective proxies of these two sources of honeybees. Observations were made during four observation periods spread over two hour intervals between 09h00 and 16h00 and were replicated five times on each parental line during each bout. Observation measurements were undertaken once on all field sites.

Seed yield

Seed yield was calculated as seed weight (g) per umbel to assess the impact of different degrees of anthophile diversity on the output of onion hybrid seed crops in South Africa. For each crop, twenty male-sterile umbels were closed with fine mesh bags before the florets opened to exclude all insect visitors for the duration of the blooming season which served as an exclusion experiment. A further 20 umbels were marked and left open for pollination, which served as an open-pollinated (control) treatment. Umbels were collected just before the seed heads were harvested, during mid-December, and stored in hessian bags. The seed heads were oven-dried (Term-O-Mat oven, Labotec, South Africa) at 50°C for three days and then the heads were processed individually to remove the individual seeds. The heads were threshed by hand and using a series of Madison test sieves (apertures used: 3.35M, 2.36M & 850µm), the seeds were separated from the remaining plant material. Seeds from each umbel were weighed using a laboratory scale accurate to three decimal places. The ratio of male-

fertile to male-sterile parent rows (relative frequency of male-fertile to male-sterile rows) were calculated for each field site and used in the analyses.

Data Analyses

Statistical analyses were done using R v.2.14.1 (R Development Core Team, 2011). A Welch two-sample t-test ("t.test" function from the "stats" package) was used to compare seed yield between open-pollinated and covered umbels. A linear mixed effects model (LMM) fit by maximum likelihood, with a Gaussian error distribution was used to test the effects of the different explanatory variables on seed yield of open pollinated umbels. The "lme" function from the "nlme" package was used (Pinheiro *et al.*, 2012). The seed weight of open-pollinated umbels was used as the response variable and was square root transformed to achieve a normal distribution. The fixed variables used in the model included: percentage natural habitat; average taxonomic distinctness as a measure of anthophile diversity; managed honeybee hive density; honeybee and non-*Apis* visitation frequency; ratio of male-fertile to male-sterile plant rows; method of irrigation; and the production region. Production region was scored as Klein Karoo or southern Karoo and was included in the model because it was found that there was a significant difference (ANOVA: $F_{1,208} = 19.10$, $P < 0.001$) in the seed yields produced by the two regions, and needed to be accounted for in the model. Pearson correlation coefficients were used to test for collinearity between the fixed variables. Sampling sites were used as the random factor in the model. Backward elimination selection procedures were followed for model simplification, starting with a model including all explanatory variables as fixed terms and dropping the least significant term, or the term which resulted in a lower AIC value, after testing the reduced model to the full model using ANOVA (Zuur *et al.*, 2009). If dropping a term did not result in a significant change ($P < 0.05$) in the model, the reduced model was retained.

RESULTS

Seed yield dependence on insect pollination

The open-pollinated or control umbels of all 12 cultivars had an average seed weight of 4.27 ± 2.08 grams per umbel (range: 0.42-14.77, N = 211) while the exclusion umbels had an average seed weight of 0.02 ± 0.08 grams per umbel (range: 0.00-0.87, N = 217) which were statistically significantly different (t = -29.61, df = 211, $P < 0.001$) (Figure 4.1).

Factors affecting seed yield

The male-fertile parental line ratio co-correlated with honeybee visitation rates and the two factors were used in separate models to determine their significance in affecting onion hybrid seed yield. (Table 4.1). Anthophile diversity and non-*Apis* visitation frequency was also co-correlated, so the factor that gave the lowest AIC value, non-*Apis* visitation frequency, was used instead. Onion hybrid seed weight was not significantly affected by the percentage of surrounding natural habitat, non-*Apis* visitation frequency, managed hive density, nor water management practices (Table 4.2). However, onion hybrid seed crops in the southern Karoo produced significantly higher yields (4.76 ± 1.87 grams/umbel ($N = 82$)) than crops grown in the Klein Karoo (3.52 ± 2.20 grams/umbel ($N = 128$)), while higher honeybee visitation frequency significantly increased seed yield. The ratio of male-fertile to male-sterile rows also significantly affected seed yield, with higher seed yields obtained from crops with higher male-fertile ratios.

DISCUSSION

Seed yield dependence on insect pollination

The average seed weight is relatively high in comparison to figures reported by other authors. Hagler and Waller (1991) reported average seed weights of 0.6-1.7 grams/umbel for five open-pollinated onion cultivars. Similarly, Williams and Free (1974) reported average seed weights of 0.3-0.7 grams/umbel for an open-pollinated block of hybrid onions. However, average seed yields of 3.2 grams/umbel were reported for a regular onion cultivar grown in Sudan (Ahmed and Abdalla, 1984), while Carlson (1974) reported average weights of 2.4-3.9 grams/umbel for open pollinated cultivars and 1.0-2.3 grams/umbel for hybrid cultivars grown in the U.S.A. The pollinator exclusion experiment showed that all the cultivars sampled in the study required insect pollination for successful production.

Factors affecting seed yield

Hybrid seed production per unit area often differs between individual crops, varieties and also between different areas of production, depending on climate, topology and soil types (Rai and Rai, 2006). The Klein Karoo and southern Karoo, the two main areas of onion hybrid seed production in the Western Cape Province, South Africa, fall both within the Succulent Karoo biome, but there are marked differences in soil type, climate and vegetation cover between the two areas. Low-lying areas in the Klein Karoo generally have nutrient rich, loamy to

clayey soils while the southern Karoo has shallow, weakly developed alkaline soils. Rainfall also differs in that the low-lying areas within the Klein Karoo usually receive 100-300 mm, and the mountainous areas receive above 1 000 mm annually (Le Maitre *et al.*, 2009) while the southern Karoo is drier, receiving 170 mm of unpredictable and highly variable rainfall annually (Cowling, 1986; Dean and Milton, 1995). Consequently, the vegetation cover in the southern Karoo is sparser than in the Klein Karoo, especially in years with low rainfall, which may result in lower wild honeybee colony densities. It was noted that very little floral resources were offered by natural vegetation in the southern Karoo at the time when the hybrid onion crops were flowering in November, which possibly forced foragers to exploit onion floral resources due to lack of alternatives. In the Klein Karoo, although the natural flowering season has mostly ended by the time the hybrid onion crops started to flower, some wild floral resources were still available. Pollinators might therefore be more numerous on crops in the southern Karoo then on crops in the Klein Karoo. Onion plants are also less susceptible to pathogens and onion diseases in the drier climate of the southern Karoo, resulting in healthier plants and consequently, higher seed yields.

Pollination is essentially a biodiversity driven ecosystem service (Millenium Ecosystem Assessment, 2005). Bio-diverse natural habitats host the organisms that pollinate natural plant populations, and often provide pollination to agricultural production as a relatively inexpensive ecosystem service. The extent and proximity of natural habitat often benefits pollinator-dependent crops in terms of improved fruit/seed set (Blanche et al., 2006; Carvalheiro et al., 2010; Greenleaf and Kremen, 2006b; Klein et al., 2012). However, in some cases it does not improve production. Chacoff *et al.*, (2008) found that, even though pollen dispersal within a grapefruit crop decreased with increased distances from the forest edge (10, 100, 500 and 1000 m), it did not affect fruit production. Additionally, Hoehn *et al.* (2008) found no significant relationship between pumpkin seed set and habitat type, ranging from natural forest, grassland, and three different management intensities of cacao agroforestry. The insignificant relationship between onion hybrid seed yield and the percentage of natural habitat surrounding the crops indicates that seed yield was not affected by the extent of natural vegetation acting as source habitat for anthophile insects. Anthophile diversity within the crops was not related to natural habitat availability and crops with low percentages of natural habitat had high levels of diversity (refer to Chapter 2). In addition, this diversity was not present on the onion flowers (refer to Chapter 3), and accordingly, anthophile diversity would not have any significant effect on seed yield.

Honeybees were by far the most abundant visitors on hybrid onion flowers (refer to Chapter 3). The significant positive relationship between honeybee visitation and seed yield confirms their effectiveness as hybrid onion pollinators. Silva and Dean (2000) showed that onion hybrid seed yield was positively correlated with the number of honeybee visits. Similar results were reported by Carlson (1974) and Ahmed and Abdalla (1984). Chandel *et al*. (2004) found that supplementing the pollinator community with managed honeybees increased onion hybrid seed yields significantly. However, it has been reported that high stocking densities of managed honeybee hives does not necessarily guarantee that honeybees will work on the hybrid onion crop (Franklin, 1970; McGregor, 1976), which is often determined by the attractiveness of the onion variety to honeybee foragers (Carlson 1974; Currah and Ockendon 1983, 1984; Lederhouse *et al*., 1972; Silva and Dean 2000).

The lack of significant relationships between seed yield and either managed hive density, or percentage natural habitat, gave no indication of whether seed yield was enhanced by the stocking rates of managed hives, or the availability of natural habitat near the crops. Similarly, honeybee visitation frequency was not significantly related to managed hive density, or to the availability of natural habitat, but rather to annual rainfall (refer to Chapter 3). The abundance of wild flowers after good seasonal rainfall may have led to both resident managed and wild honeybee colony build-up, increasing honeybee abundance on onion flowers. However, several other environmental factors also affect onion pollination, including soil fertility (Brewster, 1983; Stuart and Griffin, 1946), soil moisture (Hawthorn, 1951), temperature and humidity (Chang and Struckmeyer, 1976a, 1976b). Some factors can be controlled by farmers, who aim to control as many factors as possible that affect seed yield, such as the vernalization and spacing of mother bulbs (Hesse et al., 1979; Jones and Emsweller, 1939), the distance between parental line rows (Erickson and Gabelman, 1956), or the ratio of male-fertile to male-sterile rows (Franklin, 1958). However, several factors affecting seed yield that relates to insect visitation cannot be, or are very difficult to control. These include, weather conditions during flowering (Woyke, 1981), the attractiveness of the crop to insect visitors (Gary et al., 1977, 1972), and the presence of competing flower resources that may attract visitors away from the crop.

In terms of the effect of farm management practices, there was no significant difference in seed yield between farms that used three different irrigation methods (sprinkle, flood or drip irrigation). However, the ratio of male-fertile to male-sterile rows planted within each onion crop had a significant positive effect on seed yield. Many studies have been undertaken to

determine the optimal ratio between male-fertile and male-sterile rows within onion hybrid seed crops (Erickson and Gabelman, 1956; Franklin, 1958; Nye *et al.*, 1971; Williams and Free, 1974; Woyke and Dudek, 1983). Optimal parental line ratios allow the maximum number of male-sterile (seed producing) rows to be planted without compromising pollen transfer from the male-fertile rows. Parental line ratios used in the seed crops here, ranged from 1:2 - 1:5.5 with the most common ratio used being 1:3, which is in line with international trends (Lederhouse *et al.*, 1972; George, 1999). Nye *et al.* (1971) reported that honeybee activity and seed yield decreased as the distance from the male-fertile rows increased. Erickson and Gabelman (1956) showed that the distribution of onion pollen was logarithmic with respect to distances of male-sterile from the male-fertile rows and that seed yield decreased to half the maximum potential at 2.13 m (7 ft.) from the male-fertile lines. Unfortunately, more recent studies could not be found in the literature. The ratio of male-fertile to male-sterile plant ratios should therefore be considered as an important factor affecting the pollination of onion hybrid seed crops.

CONCLUSION

Market-related yields of onion hybrid seed were produced during the 2009 and 2010 production seasons and compared well with average seed yields reported from other global production regions, even though yields differed significantly between the two production regions used for data collection. Honeybee visitation significantly increased seed yield, but it remains unclear whether this could be attributed to managed hive density, or the availability of wild honeybees nesting in natural or semi-natural habitat near the crops. Other factors; for example onion variety attractiveness, competition from wild flowers, or annual rainfall (as shown in Chapter 3), are likely to be more important in determining honeybee visitation to onion flowers. Parental line ratios should be considered important to promote the pollination of onion hybrid seed crops.

REFERENCES

Ahmed, I.H., Abdalla, A.A., 1984. The role of honeybees as pollinators on onion (Allium cepa L.). Acta Hortic. 143, 127–132.

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.-M., 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Ann. Bot. 103, 1579–1588.

Allsopp, M.H., De Lange, W.J., Veldtman, R., 2008. Valuing insect pollination services with cost of replacement. PLoS One 3, e3128.

Blanche, K.R., Ludwig, J.A., Cunningham, S.A., 2006. Proximity to rainforest enhances pollination and fruit set in orchards. J. Appl. Ecol. 43, 1182–1187.

Bohart, G.E., Nye, W.P., Hawthorn, L.R., 1970. Onion pollination as affected by different levels of pollinator activity.

Breeze, T., Bailey, A., Balcombe, K., Potts, S.G., 2011. Pollination services in the UK: How important are honeybees? Agric. Ecosyst. Environ. 142, 137–143.

Brewster, J.L., 1983. Effects of photoperiod, nitrogen nutrition and temperature on inflorescence initiation and development in onion (Allium cepa L). Ann. Bot. 51, 429–440.

Brown, J., Wright, J.L., Kohl, R.A., 1977. Onion-seed yield and quality as affected by irrigation management. Agron. J. 69, 369–372.

Buckmann, S.L., Nabhan, G.P., 1996. The forgotten pollinators. Island Press, Washington, DC.

Cane, J.H., 1997. Ground-nesting bees: the neglected pollinator resource for agriculture. Acta Hortic. 437, 309–324.

Carlson, E.C., 1974. Onion varieties, honeybee visitations, and seed yield. Calif. Agric. 28, 16–18.

Caron, D.M., Lederhouse, R.C., Morse, R.A., 1975. Insect pollinators of onion in New York State. Hortscience 10, 273–274.

Carvalheiro, L.G., Seymour, C.L., Veldtman, R., Nicolson, S.W., 2010. Pollination services decline with distance from natural habitat even in biodiversity-rich areas. J. Appl. Ecol. 47, 810–820.

Chacoff, N.P., Aizen, M.A., 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. J. Appl. Ecol. 43, 18–27.

Chacoff, N.P., Aizen, M.A., Aschero, V., 2008. Proximity to forest edge does not affect crop production despite pollen limitation. Proc. R. Soc. B Biol. Sci. 275, 907–913.

Chandel, R.S., Thakur, R.K., Bhardwaj, N.R., Pathania, N., 2004. Onion seed crop pollination: a missing dimension in mountain horticulture. Acta Hortic. 631, 79–86.

Chang, W.N., Struckmeyer, B.E., 1976a. Influence of temperature, time of day, and flower age on pollen germination, stigma receptivity, pollen tube growth, and fruit set of Allium cepa L. J. Am. Soc. Hortic. Sci. 101, 81–83.

Chang, W.N., Struckmeyer, B.E., 1976b. Influence of temperature on seed development of Allium cepa L. J. Am. Soc. Hortic. Sci. 101, 296–298.

Clarke, K.R., Warwick, R.M., 1998. A taxonomic distinctness index and its statistical properties. J. Appl. Ecol. 35, 523–531.

Cowling, R.M., 1986. A description of the Karoo Biome Project. South African National Scientific Programmes Report No. 122. Pretoria.

Currah, L., Ockendon, D.J., 1983. Onion pollination by blowflies and honeybees in large cages. Ann. Appl. Biol. 103, 497–506.

Currah, L., Ockendon, D.J., 1984. Pollination activity by blowflies and honeybees on onions in breeders' cages. Ann. Appl. Biol. 105, 167–176.

Dean, W.R.J., Milton, S.J., 1995. Plant and invertebrate assemblages on old fields in the arid southern Karoo, South Africa. Afr. J. Ecol. 33, 1–13.

Degrandi-Hoffman, G., Watkins, J.C., 2000. The foraging activity of honey bees Apis mellifera and non-Apis bees on hybrid sunflowers (Helianthus annuus) and its influence on cross-pollination and seed set. J. Apic. Res. 39, 37–45.

Dowker, B.D., Currah, L., Horobin, J.F., Jackson, J.C., Faulkner, G.J., 1985. Seed production of an F1 hybrid onion in polyethylene tunnels. J. Hortic. Sci. 60, 251–256.

Ecosystem Assessment, M., 2005. Ecosystems and human well-being: biodiversity synthesis, Ecosystems. Washington, DC.

Erickson, H.T., Gabelman, W.H., 1954. Potential value of inbreds and F1 hybrid onions for seed production. Proc. Am. Soc. Hortic. Sci. 64, 393–398.

Erickson, H.T., Gabelman, W.H., 1956. The effect of distance and direction on cross-pollination in onions. Proc. Am. Soc. Hortic. Sci. 68, 351–357.

ESRI, 2006. ArcGIS.

Franklin, D.F., 1958. Effects on hybrid onion seed production of using different ratios of male-sterile and pollen rows. Proc. Am. Soc. Hortic. Sci. 71, 435–439.

Franklin, D.F., 1970. Problems in the production of vegetable seed, in: The Indispensable Pollinators. Arkansas Agricultural Extension Services Miscellaneous Publication 127, pp. 112–357.

Free, J.B., 1993. Insect pollination of crops. Academic Press, London, UK.

Gallai, N., Salles, J., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68, 810–821.

Gary, N.E., Witherell, P.C., Lorenzen, K., Marston, J.M., 1977. The interfield distribution of honey bees foraging on carrots, onions and safflower. Environ. Entomol. 6, 637–640.

Gary, N.E., Witherell, P.C., Marston, J.M., 1972. Foraging range and distribution of honey bees used for carrot and onion pollination. Environ. Entomol. 1, 71–78.

Greenleaf, S.S., Kremen, C., 2006a. Wild bee species increase tomato production and respond differently to surrounding land use in North California. Biol. Conserv. 133, 81–87.

Greenleaf, S.S., Kremen, C., 2006b. Wild bees enhance honey bees ' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. 103, 13890–13895.

Hagler, J.R., Wailer, G.D., 1991. Intervarietal differences in onion seed production. Bee Sci. 1, 100–105.

Hawthorn, L.R., 1951. Studies of soil moisture and spacing for seed crops of carrots and onions. United States Dep. Agric. Circ. 892, 26p.

Heard, T.A., Exley, E., 1994. Diversity, abundance and distribution of insect visitors to macadamia flowers. Environ. Entomol. 23, 91–100.

Hepburn, H.R., Radloff, S.E., 1998. Honeybees of Africa. Springer Verlag, Berlin.

Hesse, P.S., Vest, G., Honma, S., 1979. The effect of four storage treatments on seed yield components of three onion inbreds. Sci. Hortic. (Amsterdam). 11, 207–215.

Hilton-Tailor, C., Le Roux, A., 1989. Conservation status of the Fynbos and Karoo biomes, in: Huntley, B.J. (Ed.), Biotic Diversity in Southern Africa. Oxford University Press, Cape Town, pp. 202–223.

Hoehn, P., Tscharntke, T., Tylianakis, J.M., Steffan-Dewenter, I., 2008. Functional group diversity of bee pollinators increases crop yield. Proc. R. Soc. B-Biological Sci. 275, 2283–2291.

Howlett, B.G., Donovan, B.J., McCallum, J.A., Newstrom, L.E., Teulon, D.A.J., 2005. Between and within field variability of New Zealand indigenous flower visitors to onions. New Zeal. Plant Prot. 58, 213–218.

Johannsmeier, M.F., 2001. Beekeeping in South Africa, Third edit. ed. Agricultural Research Council-Plant Protection Research Institute, Handbook No. 14.

Jones, H.A., 1923. Pollination and self-fertility in the onion. Proc. Am. Soc. Hortic. Sci. 20, 191–197.

Jones, H.A., Emsweller, S.L., 1939. Effect of storage, bulb size, spacing, and time of planting on production of onion seed. Bull. 628, Univ. California, Berkeley.

Kearns, C.A., Inouye, D.W., 1993. Techniques for pollination biologists. University Press of Colorado, Niwot.

Klein, A.-M., Brittain, C., Hendrix, S.D., Thorp, R., Williams, N.M., Kremen, C., 2012. Wild pollination services to California almond rely on semi-natural habitat. J. Appl. Ecol. 49, 723–732.

Klein, A.-M., Steffan-Dewenter, I., Tscharntke, T., 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proc. R. Soc. B Biol. Sci. 270, 955–961.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274, 303–313.

Kremen, C., Williams, N.M., Bugg, R.L., Fray, J.P., Thorp, R.W., 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecol. Lett. 7, 1109–1119.

Kremen, C., Williams, N.M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. Proc. Natl. Acad. Sci. 99, 16812–16816.

Le Maitre, D.C., Colvin, C., Maherry, A., 2009. Water resources in the Klein Karoo: the challenge of sustainable development in a water-scarce area. S. Afr. J. Sci. 105, 39–48.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1968. Onion pollination in New York. New York's Food Life Sci. Bull. 1, 8–9.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1972. Distribution and behavior of honey bees on onion. Environ. Entomol. 1, 127–129.

Levy, D., Ben-Herut, Z., Albasel, N., Kaisi, F., Manasra, I., 1981. Growing onion seeds in an arid region: drought tolerance and the effect of bulb weight, spacing and fertilization. Sci. Hortic. (Amsterdam). 14, 1–7.

Long, R.F., Morandin, L., 2011. Low hybrid onion seed yields relate to honey bee visits and insecticide use. Calif. Agric. 65, 155–159.

MacGillibray, J.H., 1948. Effect of irrigation on the yield of onion seed. Proc. Am. Soc. Hortic. Sci. 51, 423–427.

Mann, L.P., Woodbury, G.W., 1969. The effect of flower age, time of day and variety on pollen germination of onion, Allium cepa L. J. Am. Soc. Hortic. Sci. 94, 102–104.

Mayer, D.F., Lunden, J.D., 2001. Honey bee management and wild bees for pollination of hybrid onion seed. Acta Hortic. 561, 275–278.

McGregor, S.E., 1976. Common vegetables for seed and fruit, in: McGregor, S.E. (Ed.), Insect Pollination of Cultivated Crop Plants. USDA, Washington.

Moffett, J.O., 1965. Pollinating experimental onion varieties. Am. Bee J. 105, 378.

Moll, R.H., 1953. A study of receptivity in the onion flower Allium cepa Linn. and some factors affecting its duration. University of Idaho.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., de Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nye, W.P., Waller, G.D., Waters, N., 1971. Factors affecting pollination of onions in Idaho during 1969. J. Am. Soc. Hortic. Sci. 96, 330–332.

Parker, F.D., 1982. Efficiency of bees in pollinating onion flowers. J. Kansas Entomol. Soc. 55, 171–176.

Pathak, C.S., 2000. Hybrid seed production in onion. J. New Seeds 1, 89–108.

Pinheiro, J.C., Bates, D., DebRoy, S., Sarkar, D., Authors, E., R-core, 2012. Package "nlme."

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353.

R, D.C.T., 2011. R: A language and environment for statistical computing.

Rader, R., Howlett, B.G., Cunningham, S. a., Westcott, D. a., Edwards, W., 2012. Spatial and temporal variation in pollinator effectiveness: do unmanaged insects provide consistent pollination services to mass flowering crops? J. Appl. Ecol. 49, 126–134.

Rai, N., Rai, M., 2006. Heterosis breeding in vegetable crops. New India Publishing Agency, New Delhi.

Ribbands, C.R., 1951. The flight range of the honey-bee. J. Anim. Ecol. 20, 220–226.

Saeed, S., Sajjad, A., Kwon, O., Kwon, Y.J., 2008. Fidelity of Hymenoptera and Diptera pollinators in onion (Allium cepa L.) pollination. Entomol. Res. 38, 276–280.

Sajjad, A., Saeed, S., Masood, A., 2008. Pollinator community of onion (Allium cepa L.) and its role in crop reproductive success. Pak. J. Zool. 40, 451–456.

Schittenhelm, S., Gladis, T., Rao, V.R., 1997. Efficiency of various insects in germplasm regeneration of carrot, onion and turnip rape accessions. Plant Breed. 116, 369–375.

Shanmugasundaram, S., 1998. Onion cultivation and seed production. Shanhua, Tainan, AVRDC, Taiwan.

Silva, E.M., Dean, B.B., 2000. Effect of nectar composition and nectar concentration on honey bee (Hymenoptera: Apidae) visitations to hybrid onion flowers. J. Econ. Entomol. 93, 1216–1221.

Southwick, E.E., Southwick, L., 1992. Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. J. Econ. Entomol. 85, 621–633.

Steffan-Dewenter, I., Potts, S.G., Packer, L., 2005. Pollinator diversity and crop pollination services are at risk. Trends Ecol. Evol. 20, 651–652.

Stuart, N.W., Griffin, D.M., 1946. The influence of nitrogen nutrition on onion seed production in the greenhouse. Proc. Am. Soc. Hortic. Sci. 48, 398–402.

Vaissière, B.E., Cambon, G., Morison, N., Broucqsault, L.M., 2001. Pollination effectiveness of the airborne pollen flow in onion, Allium cepa, an entomophilous species. Apidologie 32, 186–187.

Van der Walt, W.J., 1999. Changing face of South African seed trade. J. New Seeds 1, 81–97.

Van der Walt, W.J., 2002. A review of the South African seed industry. J. New Seeds 4, 57–70.

Walker, M.K., Howlett, B.G., Wallace, A.R., Mccallum, J.A., Teulon, D.A.J., 2011. The diversity and abundance of small arthropods in onion, Allium cepa, seed crops, and their potential role in pollination. J. Insect Sci. 11, Check on the 1st page for citing instructions.

Waller, G.D., Carpenter, E.W., Ziehl, O.A., 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. J. Am. Soc. Hortic. Sci. 97, 535–539.

Waller, G.D., Gary, N.E., Chester Jr, S.T., Karim, M.S., Martin, J.H., Vaissière, B.E., 1985. Honeybee colony populations and foraging rates on onion seed-fields in Arizona. J. Apic. Res. 24, 93–101.

Walsh, R.S., 1965. Pollination of onion plants by honeybees. New Zeal. Beekeep. 27, 18–20.

Westerkamp, C., Gottsberger, G., 2000. Diversity Pays in Crop Pollination. Crop Sci. 40, 1209–1222.

Westerkamp, C., Gottsberger, G., 2001. Pollinator Diversity is Mandatory for Crop Diversity. Acta Hortic. 561, 309–316.

Westerkamp, C., Gottsberger, G., 2002. The costly crop pollination crisis, in: Kevan, P.G., Imperatriz-Fonseca, V.L. (Eds.), Pollinating Bees - the Conservation Link Between Agriculture and Nature. Ministry of Environment, pp. 51–56.

Wilkaniec, Z., Giejdasz, K., Proszynski, G., Zdzislaw, W., Giejdasz, J., 2004. Effect of pollination on onion seeds under isolation by the mason bee (Osmia rufa L.) (Apoidea, Megachilidae) on the setting and quality of obtained seeds. J. Apic. Sci. 48, 35–41.

Williams, I.H., Free, J.B., 1974. The pollination of onion (Allium cepa L.) to produce hybrid seed. J. Appl. Ecol. 11, 409–417.

Winfree, R., Williams, N.M., Dushoff, J., Kremen, C., 2007. Native bees provide insurance against ongoing honey bee losses. Ecol. Lett. 10, 1105–1113.

Witter, S., Blochtein, B., 2003. Effect of pollination by bees and other insects on the production of onion seeds. Pesqui. Agropecuária Bras. 38, 1399–1407.

Woyke, H.W., 1981. Some aspects of the role of the honeybee in onion seed production in Poland. Acta Hortic. 111, 91–98.

Woyke, H.W., Dudek, M.M., 1983. The influence of distance between male-sterile and male-fertile rows on the number of honeybees visiting the flowers and on the seed yield of hybrid onion, in: Proceedings of the Vth International Symposium on Pollination. pp. 123–128.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

Table 4.1: Pearson correlation coefficients between continuous explanatory variables that were used in the linear mixed effects model. A cut-off correlation of 0.60 was used as selection criteria, with those correlations rounding to or above this value printed in bold.

Fixed variables	Natural habitat (%)	Anthophile Δ*	Hive density	Honeybee VF	non-*Apis* VF
Anthophile Δ*	0.20				
Hive density	0.16	-0.10			
Honeybee visitation frequency	-0.50	-0.13	0.01		
non-*Apis* visitation frequency	0.29	**0.57**	0.24	-0.37	
Male-fertile line ratio	-0.06	-0.33	-0.03	**0.68**	-0.27

Δ* = average taxonomic distinctness, VF = visitation frequency

Table 4.2: Results of the linear mixed effects model analyses of seed weight per open-pollinated umbel for 13 hybrid onion field sites. The *t*-values from likelihood ratio tests and the direction of the relationships (+/−) are shown for the variables in the two final models. Blank spaces represent dropped variables following stepwise deletion. Number of observations = 210, for 13 farms.

	Model	AIC	SD$	Natural habitat (%)	Anthophile Δ*	Hive density (/ha)	Honeybee VF / Male-fertile ratio	non-*Apis* VF	Irrigation Method	Production Region
Honeybee VF	M1	231.6	0.15			-1.60 (-)	3.57* (+)	-1.72 (-)	-1.26 f < d -2.31 s < d	4.89** sK > KK
	M2	232.4	0.14	0.31 (+)		-1.60 (-)	3.56* (+)	-1.75 (-)	-1.27 f < d -2.15 s < d	4.33** sK > KK
Male-fertile ratio	M1	235.03	0.26				2.25* (+)			3.37** sK > KK
	M2	234.52	0.23				2.28* (+)	-1.65 (-)		4.01** sK > KK

(***$P < 0.001$, **$P < 0.01$, *$P < 0.05$), Δ* = average taxonomic distinctness, VF = visitation frequency. $ Standard deviation of random factor. † Method of irrigation included drip (d), flood (f), and sprinkle (s) irrigation. ‡ The production regions were the Klein Karoo (KK) and southern Karoo (sK).

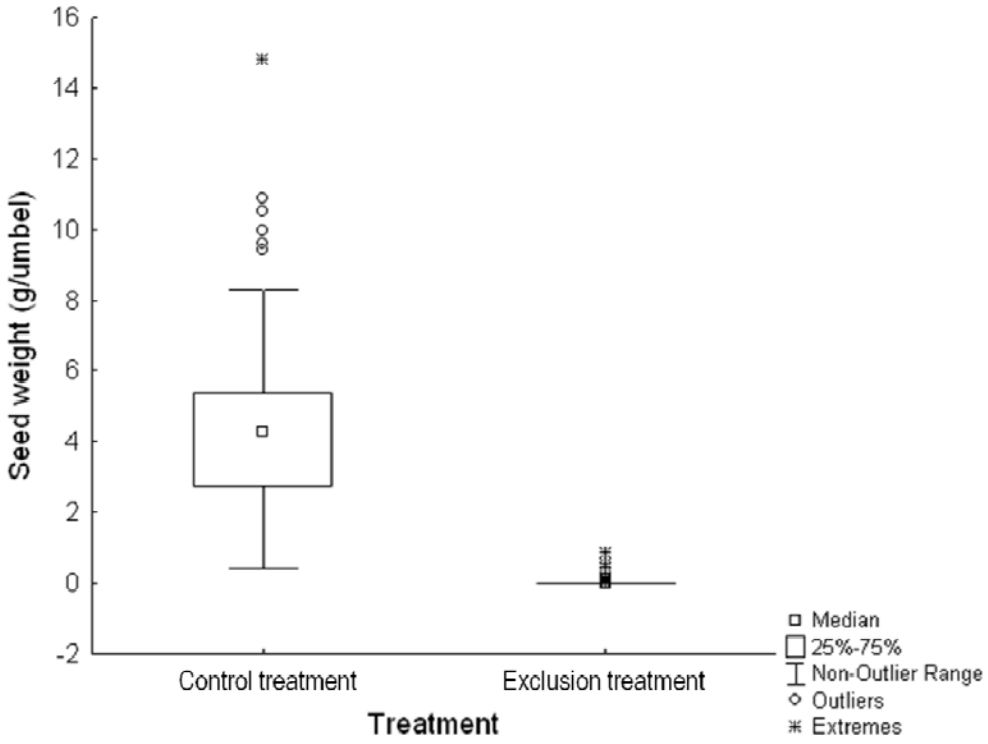


Figure 4.1: Seed yield of umbels exposed to continuous pollination during flowering (control treatment) and umbels where insect visitors have been excluded during flowering (exclusion treatment).

5

HONEYBEE FORAGING PATTERNS ON ONION HYBRID SEED CROPS IN SOUTH AFRICA: FLORISTIC AND BIOTIC FACTORS THAT MIGHT INFLUENCE CROSS-POLLINATION

ABSTRACT

Cross-pollination of onion hybrid seed crops is highly dependent on insect activity to carry pollen from the pollen producing male-fertile to the seed-producing male-sterile flowers. However, hybrid onion parental lines may differ in their attractiveness to foraging honeybees, the main pollinators of onion flowers. This chapter sets out to evaluate floral characteristics that cause honeybees to discriminate between parental lines. In addition, honeybee foraging patterns within and between parental line flowers were recorded, especially in the context of increased movement between parental lines induced through behavioural interactions with other non-*Apis* flower-visitors, and which could possibly suggest an important ecosystem service obtained from the diversity of flower-visiting species foraging on onion umbels. Nectar volume and sugar concentrations were compared between parental lines and varieties, as nectar is the main attractant for honeybees and offered by both parental lines. In addition, floral scents is a powerful foraging cue used by honeybees to identify rewarding flowers and practice floral constancy. Therefore, volatile scent compounds were sampled to test for any differences in floral scent profiles between parental lines and varieties that might cause honeybees to discriminate between them when foraging. Results show that the male-fertile parental lines secreted significantly more nectar, while the nectar from male-sterile lines was generally more concentrated. The floral scent profiles of the parental line umbels differed significantly, further differentiating parental line characteristics. Honeybees showed marked discrimination between parental lines when foraging. In addition, very few interactions were recorded between foraging honeybees and non-*Apis* visitors that caused honeybees to forage on the opposite parental line in a single foraging bout. Although it has been shown in the previous chapter that honeybee visitation is the principal means of successful pollination of onion hybrid seed crops, the mechanism and degree of honeybee pollen transfer remains unclear with regard to the findings reported here. In addition, non-*Apis* visitors were limited in numbers and do not seem to cause honeybee movement between parental lines and thus, for South Africa, does not contribute to hybrid onion pollination as an ecosystem service.

INTRODUCTION

Hybrid varieties produce plant and product uniformity and hybrid onion cultivars are a dominant trend in commercial onion breeding (Brewster, 2008; Wills and North, 1978). Cytoplasmic male sterility was first discovered in the onion (*Allium cepa* L.) by Jones & Clarke (1943) which led to the development of F_1 hybrid onion cultivars through the establishment of cross breeding parental lines (George, 1999). Three parental lines are used and maintained for F_1 hybrid cultivars namely A, B and R. Line A is the male-sterile parent with deformed or suppressed anthers that result in the absence of pollen (Wills & North, 1978). Line B is the maintainer line used to maintain male-sterility in line A, and the restorer line (R) is the male-fertile parent with anthers that produce viable pollen (Shanmugasundaram, 1998; Pathak, 2000). Hybrid seed is produced when line A and R are crossed (Shanmugasundaram, 1998; Pathak, 2000).

Although onion is largely self-compatible, individual onion flowers are protandrous which limits self-pollination to a degree (Free, 1993). Insects are integral for the transfer of pollen between individual onion flowers. Onions have a bee factor estimated to be approximately 95% (Johannsmeier, 2005), which assumes no pollination to occur in the absence of insect pollinators. Both the breeding and production of onion hybrid seed is therefore totally dependent on insect pollen vectors to carry pollen from male-fertile to male-sterile plants to bring about pollination and ensure the production of viable seeds (Brewster, 2008). Honeybees, which can be effectively managed and supplied in great numbers to pollinate crops (Free, 1993), are considered the most abundant and important pollinators of onion seed crops (Benedek, 1977; Howlett *et al.*, 2005; Nye *et al.*, 1973; Witter and Blochtein, 2003; see also Chapter 4).

Apart from onion flowers being relatively unattractive to honeybees (Gary *et al.*, 1972; Gary *et al.* 1977b), honeybees have also been found to show preference between male-fertile and female-sterile umbels and will forage selectively between male-fertile and male-sterile umbels within a onion hybrid seed crop. Several authors have found higher numbers of honeybees foraging on male-fertile rows than on male-sterile rows (Williams and Free, 1974; McGregor, 1976; Woyke, 1981; Parker, 1982; Silva, 1998; Mayer and Lunden, 2001). In contrast, observations made by Dowker *et al.* (1985) showed that honeybees visited, on average, more flowers and spent more time per umbel on male-sterile plants than on male-fertile plants. These observations suggest discriminating behaviour by honeybees when

visiting male-fertile and male-sterile umbels and which may be linked to honeybees adapting their foraging behaviour to those lines with greater rewards (*sensu* Waddington, 1980). Such behaviour of honeybees being more attracted to one onion parental line above the other within a crop field, would be detrimental to cross-pollination, a key requisite for hybrid-seed production.

Wills and North (1978) suggested that the discrimination between parental lines by pollinators could be reduced by selection during breeding programs to minimize inconsistent floral attributes. According to Waller (1972) the following factors should be considered when investigating selective foraging by pollinators: the innate and learnt behaviour of the foragers; the colour and form of the flowers; floral odours; nectar and pollen characteristics; and possible interrelationships between these factors. It is well known that honeybees practice floral constancy when foraging, in that individual foragers generally visit flowers of the same plant species in single foraging bouts (Grant, 1950). However, the umbels and florets of hybrid onion parental lines are uniform in shape and colour, and therefore are unlikely to be a discriminating factor between parental lines. However, differences in the characteristics of nectar (the main food resource for honeybees from onions), and odour (an important foraging cue) have not been investigated for South African hybrid onions and will be assessed in this chapter.

Onion Nectar

Sugar is the main component of all nectars and its concentration determines nectar viscosity (Corbet, 1978). Onion nectar is usually highly concentrated, with sugar concentrations exceeding 40% (Free, 1970; Hagler *et al.*, 1990; Kumar and Gupta, 1993; Lederhouse *et al.*, 1972; Waters, 1972), remaining fairly constant over the lifetime of the flowers (Silva *et al.*, 2004). Silva and Dean (2000) found that sucrose was absend and the proportion of fructose (58%) and glucose (42%) were fairly constant in the nectar of nine onion cultivars. Their findings concur with the general rule of hexose-dominated nectars in shallow flowers with unprotected nectaries (Percival, 1961).

In some cases, nectar also contains small amounts of other constituents that contribute to its aroma and taste (Baker, 1977; Free, 1970). Among these are amino acids, which are universally present (Baker, 1977), volatile oils, polysaccharides, proteins, enzymes, ions and alkaloids (Percival, 1965; Baker and Baker, 1982; Nicolson and Thornburg, 2007). Hagler (1990) and Waller *et al.* (1972) found that the potassium levels in onion nectar were

approximately ten times higher than in nectar from competing flora, with levels varying between 3,600-13,000 ppm (parts per million), and that the nectar of competing flora with lower potassium levels were more attractive to honeybees than onion nectar. However, Silva and Dean (2000) found no significant correlation between nectar potassium concentrations and the attractiveness of hybrid onion flowers to honeybees, and potassium concentration varied significantly between parental lines and between years. They did not offer any explanations for their findings.

Over the lifetime of the onion flower, peak nectar production occurs when the pollen is shed (Silva *et al.*, 2004). Nectar secretion peaks around mid- to late-afternoon and again during late-evening (Silva *et al.*, 2004). Nectar collecting insects should move freely between the two hybrid parental lines to bring about pollination because male-sterile florets do not produce pollen (Benedek, 1977). It is therefore important that the flowers of both parental lines offer equally attractive nectar sources. Previous comparisons, however, have shown that there are often differences in the nectar volume and concentration offered by each hybrid parent (Lederhouse *et al.*, 1968; Ali *et al.*, 1984; Silva, 1998).

Umbel scent

Bees recognise flowers by their colour, shape and odour (Free, 1993). Bees are attracted to flowers from a distance primarily by the shape and colour of the flower, but at close range scent provides the stimulus to alight (Backhaus, 1993; Free, 1993). The sense of smell is highly developed in bees and they associate high-value forage sources with a particular scent or mixture of scents (Joerges *et al.*, 1997; Ribbands, 1955). Odour is an important foraging cue used by bees to practice floral constancy (Chittka and Thomson, 1999). Floral scent is an important olfactory cue that evolved to attract pollinators, and usually consists of a complex blend of volatile chemical compounds which may belong to several different chemical classes (Knudsen *et al.*, 1993).

Free (1969) reported that it is possible to attract honeybees to a crop by incorporating the odour of the target crop into the colony's food stores. In an attempt to attract more honeybees to an onion crop, Silva *et al.* (2003) conditioned honeybees to associate onion floral odour with a reward, using a 30% sucrose solution scented with a 0.2% solution of onion flower volatiles. However, they found that conditioned bees did not prefer onion flowers above two competing food sources, and concluded that preconditioning bees using

onion scent would not alter honeybee behaviour to an extent that it would become an economically viable option.

Waters (1972) described in detail the behaviour of individual honeybees when foraging for nectar from onion umbels. He observed a definite reluctance to land on the flowering umbels. The honeybees would rather approach an umbel and hover in front of it, testing the florets with their antennae. In some cases the honeybee would not land and would move on to the next umbel. Waters (1972) hypothesised that honeybees searched for newly opened flowers containing diluted nectar. It therefore appears that honeybees use floral odour cues to discriminate between the umbels of onion parent plants. This behaviour restricts opportunities for inadvertent contact with the floral reproductive parts on umbels of the opposite parent, thereby limiting pollination.

Honeybee movement between parental lines and anthophile interaction

Interactions between foraging honeybees and other pollinator insects in hybrid seed crop fields have been found to increase pollination by encouraging the movement of honeybees between hybrid parental lines and thereby affecting the transfer of pollen. For example, DeGrandi-Hoffman and Watkins (2000) suggested that a combined honeybee and non-*Apis* bee population might result in better pollination of hybrid sunflowers than either population alone, even though the average number of honeybees counted daily was approximately seven times larger than the total number of non-*Apis* bees counted. They ascribed this to the positive correlation of both seed set and the amount of sunflower pollen on the bodies of honeybees foraging on male-sterile rows with the size of the non-*Apis* bee population. Similar results have also been seen for other hybrid seed crops, with Greenleaf and Kremen (2006) finding that behavioural interactions between honeybees and non-*Apis* bees in hybrid sunflower seed crops increased pollination up to 5-fold in some plots. These interactions caused honeybees to move more readily from male-fertile to male-sterile rows, thus increasing visitation and pollen transfer rates. The possibility for such interactions in South African hybrid onion crops appears viable due to the presence of non-*Apis* visitors on the flowers, though in low numbers (Chapter 3) and the rich anthophile diversity found within crop fields (Chapter 2). This non-*Apis* component may offer benefits to the farmers through increasing overall movement of honeybees between parental lines; however, this has not been tested to date in South Africa.

Aims

The central aim of this chapter is to describe honeybee behaviour when foraging on male-fertile and male-sterile rows within onion hybrid seed crops grown in the semi-arid regions of South Africa. Factors that could contribute to the discrimination between parental lines by honeybees are evaluated by testing for differences in nectar volume and sugar concentration between the parental lines, and comparing the chemical composition of volatile compounds emitted from male-fertile and male-sterile umbels. These possibly discriminating factors are also compared between the different hybrid onion varieties. I also test the hypothesis that interactions between honeybees and other flower-visiting insects on hybrid onion umbels could increase the movement of honeybees between male-fertile and male-sterile rows, and therefore benefit pollen transfer and seed yield and which could possibly signify a valuable ecosystem service from a diversity of pollinators present in onion hybrid seed crops.

METHODS

Study Sites

Eight farms growing onion hybrid seed were used for data collection during 2009, 2010 and 2011, from late October through November. The farms (09JO, 09JR, 10DCF, 10FdT, 10BvA, 10BLL, 11LvdW, 11PS) were situated in the Klein Karoo and southern Karoo regions in the Western Cape (refer to Table A.1 and Figure A.1 in Appendix A for additional information on the sites). These areas are climatically well suited for the production of onion seed, which requires low humidity and mild cool temperatures during the initial growth phase, followed by increased temperatures later on to induce flowering (Shanmugasundaram, 1998). It proved to be very difficult to nearly impossible to obtain true or definite cultivar information from the participating seed companies and seed growers due to confidentiality of varietal crosses used to create hybrids and competition between seed companies. Therefore, distinguishing varietal differences between the farms was based solely on coded information given by seed companies i.e. farm sites were able to be classed based on which variety was grown, but no other information on the variety or crosses used were given. Data were collected during peak flowering periods (50% florets open), on warm, sunny days with temperatures above 25˚C, and wind speeds of below 10 km/hour.

Onion Nectar

Nectar volume and sugar concentration were measured for 5 onion hybrid seed varieties (coded as E, F, I, P & Q, refer to Table A.1 in Appendix A) to determine whether there were any differences in nectar rewards offered by the two parental lines and also between varieties. Between 7 and 11 of both male-fertile and male-sterile umbels per variety were closed with fine mesh bags before 8h00 on sampling days, in order to exclude insect visitors. For each umbel, nectar volumes were measured from at least six individual florets with one whorl of dehiscing stamens, using the length of the nectar columns in 1 and 2 µl micro-capillary tubes (Drummond Scientific). A hand-held refractometer (40 – 85%: Bellingham & Stanley, Tunbridge Wells, UK) adjusted for small volumes was used to measure the sugar concentration of the nectar from each floret immediately after measurement of its volume. All measurements were taken between 13h00 and 18h00 in the afternoon to allow for sufficient nectar to accumulate during morning hours.

I-buttons (Fairbank technology, USA) were used to measure temperatures during nectar sampling for three different varieties (E, F & I). I-buttons were inserted in the mesh bags enclosing the umbels and suspended in mid-air to measure ambient temperature. All I-buttons were positioned to avoid direct exposure to sunlight as far as possible. The I-buttons were set to record temperatures (˚C) at 10 minute intervals during the sampling day. A hand-held weather tracker (Kestrel 4000, Nielsen-Kellerman U.S.A.) was used for ambient temperature measurements when nectar samples were collected for two varieties (P and Q) because I-buttons were not available. Recordings were made only during nectar sampling from 13h00 to 18h00.

Umbel scent

Headspace sampling was used to collect volatile chemical compounds from the umbels of 3 onion hybrid seed varieties (coded as E, F and I, refer to Table A.1 in Appendix A) grown on 4 farms to determine whether there were any notable differences in scent compounds between parental lines and also between varieties. Polyacetate bags (Nalo Bratfolie Kalle GmbH-Germany) were placed over the umbels just prior to sampling to concentrate the volatile compounds. All samples were taken between 10h00 and 16h00 on two consecutive days. Air from inside the bags were suctioned for 30 minutes into an absorbent chromatoprobe trap containing 2 mg of a 1:1 mixture of Tenax TA® (Alltech Associated, USA) and graphitized carbon (Carbotrap™, Supelco, USA), using a portable battery-operated pump (Spectrex

Personal Air Sampler PAS 500) calibrated at 200 mL min^{-1}. Air samples were simultaneously collected from empty polyacetate bags as controls to identify background contamination. The trap samples were stored at -20 ˚C in sealed vials until analysis.

The graphitized carbon is highly retentive, and small quantities can be used due to its high absorbing capacity (Millar and Sims, 1998; Tholl and Rose, 2006), thus allowing for longer sampling time at a higher flow rate. The adsorbent Tenax TA used in the study is commonly used to trap volatile compounds and has a high thermal stability of up to 350 ˚C which allows for thermal desorption in gas chromatography (GC) analysis (Tholl and Rose, 2006).

Volatile samples were analyzed using a coupled Varian 3800 gas chromatograph (GC) (Varian Palo Alto, California, USA) and a Varian 1200 mass spectrometer (MS). The GC was equipped with an Alltech EC-WAX column (carbowax column) of 30 m x 0.32 mm internal diameter x 0.25 µm film thickness (Alltech Associates Inc., Deerfield, Illinois, USA). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. Traps containing the absorbent and volatiles were placed in a Varian 1079 injector by means of a chromatoprobe fitting and thermally desorbed. After a 3 minute hold at 40 ˚C the GC oven was ramped up to 240 ˚C at 10 ˚C min^{-1} and held there for 12 minutes. Compound identification was carried out using the NIST05 mass spectral library and comparisons with retention times of chemical standards, where available, as well as comparisons between calculated Kovats retention indices and those published in the literature. A homologous series of alkanes (C8-C20) was used to determine Kovats retention indices. All reference compounds used for retention time comparisons were obtained from Sigma Aldrich Inc. GmbH, Germany. Compounds present at higher or similar percentages in controls were considered as contaminants and excluded from the analysis.

Honeybee movement between parental lines and anthophile interaction

Foraging honeybees were observed during four data collection periods spread over 2 hour intervals between 09h00 and 16h00 on 7 hybrid onion varieties (including E, F, I-M). Six to twelve individual foraging honeybees were identified on male-fertile umbels during each observation period and followed for up to 15 consecutive visits to umbels or until the honeybee was lost. Parent identity (male-fertile/male-sterile) was recorded for every umbel a honeybee visited. To determine whether behavioural interactions between foraging honeybees and other insect visitors led to increased honeybee movement between the parental lines, any interactions that occurred were noted and the insect species involved were

recorded. After an interaction took place the foraging honeybee was then followed for four more visits to umbels, noting whether the umbels were male-fertile or male-sterile. The same procedure was repeated on male-sterile umbels during each sampling period.

Data Analysis

Onion Nectar

Factorial analysis of variance (ANOVA) was performed using Statistica 10 (StatSoft Inc., USA) to test for significant differences in nectar volumes and sugar concentrations between male-fertile and male-sterile umbels and between varieties (main effects), and interactions between the two. Student's two-tailed t-tests were used to do pair-wise tests for significant differences ($P < 0.05$) between groups, using the 't.test' function from the 'stats' package in R (R Development Core Team, 2011). Student's two-tailed t-tests were also used to test for significant differences ($P < 0.05$) between ambient and mesh bag temperatures.

Umbel scent

Multivariate techniques are the most suitable to visualize and analyse differences in fragrance chemistry with characteristic locations in "odour space", since fragrance cannot be categorized along any single axis of physical properties (Raguso, 2001). Relative proportions of the different compounds in each sample were used for analyses. PRIMER v6.1.14 (PRIMER-E Ltd., UK) was used to analyse and compare scent profiles of male-fertile and male-sterile umbels and for each hybrid onion variety. Non-metric multi-dimensional scaling (MDS), based on Bray-Curtis similarities of square root-transformed data, were used to detect similarities among the samples of the different groups in low-dimensional space. Two-way crossed analysis of similarities (ANOSIM) was used to further test for any significant differences in scent profiles between the groups, using 10 000 permutations (factor A: Variety; factor B: Parental line). The resulting test statistic R was taken as a relative measure of separation between defined groups, based on mean ranks between and within groups (0 = no separation and 1 = complete separation (Clarke and Gorley, 2006). The similarity percentages (SIMPER) routine in PRIMER was used to examine the contributions of individual compounds to the separation of the groups. The BEST/BVSTEP routine in PRIMER (Clarke and Warwick, 1998) was used to select the smallest possible subset of volatile compounds which, in combination, would describe most of the patterns observed in the full dataset. A

Spearman rank correlation was used to compare the Bray-Curtis similarity matrices of the full set and subset of compounds.

Honeybee movement between parental lines and anthophile interaction

Honeybee movement between hybrid onion umbels was scored as 'hops' and classified as hops between male-fertile and male-fertile; male-fertile and male-sterile; male-sterile and male-sterile; and male-sterile and male-fertile umbels. The total numbers of hops within each of these categories were reported for honeybees followed on male-fertile and also on male-sterile umbels, along with the percentage of hops made within each category from the total number of hops recorded. Chi-square tests were used to test for significant differences in the number of hops made between the parental lines. The total number and the percentage of interactions that occurred in all observations between honeybees and other insect visitors are reported.

RESULTS

Onion Nectar

Mean ambient temperatures fluctuated between 25 and 35 ˚C in the period between 10h00 and 17h00 on the days of nectar sampling (Figure 5.1a). The mean temperatures measured in the mesh bags (25.96 $\pm$ 3.32 °C) were significantly lower than the mean ambient temperatures (28.44 $\pm$ 4.20 °C) measured throughout the day ($F = 35.37$, $df = 1$, $P < 0.001$) (Figure 5.1b). The difference in mean ambient and mesh bag temperatures ranged from 2.5 ˚C up to approximately 5 ˚C between 11h00 and 12h00.

Nectar production varied greatly from 0.03 µl/floret to 1.15 µl/floret. Nectar volume differed significantly between parental lines (Table 5.1, $F = 128.71$, $df = 1$, $P < 0.001$). Male-fertile florets had significantly more nectar than male-sterile florets for all five varieties (E: $t = -4.57$, $df = 97$, $P < 0.001$; F: $t = -8.42$, $df = 134$, $P < 0.001$; I: $t = -3.95$, $df = 112$, $P < 0.001$; P: $t = -2.98$, $df = 118$, $P = 0.003$; Q: $t = -5.46$, $df = 118$, $P < 0.001$). Nectar volume also differed significantly between varieties (see Figure 5.2, $F = 19.37$, $df = 4$, $P < 0.001$). The interaction between parental line and variety was also significant ($F = 4.71$, $df = 4$, $P < 0.001$).

Generally, the nectar in male-sterile florets was significantly more concentrated than in male-fertile florets (Table 5.1, $F = 12.6$, $df = 1$, $P < 0.001$). Within varieties E, P and Q, male-

sterile florets had significantly higher sugar concentrations than male-fertile florets (E: t = 4.41, df = 92, $P < 0.001$; P: t = 2.64, df = 82, $P = 0.01$; Q: t = 2.93, df = 106, $P = 0.004$), while concentrations did not differ within varieties F (t = -1.14, df = 101, $P = 0.26$) and I (t = -1.25, df = 103, $P = 0.22$). Sugar concentrations also differed significantly between varieties (see Figure 5.3, F = 44.67, df = 4, $P < 0.001$). The interaction between parental line and variety in terms of sugar concentration was also significant (F = 11.23, df = 4, $P < 0.001$).

Umbel scent

The volatile scent compounds emitted by male-fertile and male-sterile hybrid onion umbels are listed in Table 5.2. In total, 41 compounds were detected in all the odour samples and 40 were identified. All the compounds were common to both parents, while 8 compounds were not common to all four varieties. The identified compounds included 19 fatty acid derivatives (one unsaturated hydrocarbon, one aliphatic acid, six aldehydes, three ketones and eight alcohols), 12 benzenoids, one lactone and 8 sulphur-containing compounds. The most dominant compound class was benzenoids (71.9%), followed by aldehydes (10.3%) and alcohols (8.6%). Benzaldehyde was by far the most abundant compound, contributing 61.13% to the total abundance, followed by heptanal (4.75%), benzyl alcohol (4.68%), and hexanal (3.37%). The most abundant sulphur-containing compound was dipropyl disulphide (2.42% of total abundance).

An MDS analysis (2D stress value = 0.16) showed no separation in the chemical compositions of the parental lines (Figure 5.4), or the varieties (Figure 5.5). However, a stress value of 0.16 is high enough to suspect that the ordination of the data cloud might be misleading (Clarke and Gorley, 2006). The ANOSIM analysis showed significant differences between the parental lines (R = 0.323, $P < 0.05$) and between the varieties (R = 0.404, $P < 0.05$). Pair-wise tests showed that significant differences in compound compositions occurred between different varieties (E vs. I: R = 0.54, $P = 0.01$; I vs. F1: R = 0.71, $P = 0.01$; I vs. F2: R = 0.36, $P = 0.01$; E vs. F1: R = 0.48, $P = 0.01$; E vs. F2: R = 0.25, $P = 0.02$), but not between the two same varieties grown on different farms (F1 vs. F2: R = 0.13, $P = 6.1$).

Within-group similarities and between-group dissimilarities were not caused by the presence or absence of single compounds, but seemed to result from many small contributions from a large number of compounds. About 50% of the similarities within the groups were described by up to 5 compounds per group (Table 5.3), while small contributions from most compounds added to 100% similarities within groups. Thirteen

compounds were identified that, in combination, represented a subset that closely reflected the patterns observed in the full dataset. These compounds are indicated in bold in Table 5.2.

In terms of the behaviour of foraging honeybees, it was noted that when a honeybee foraged on umbels of one parental line and then came across an umbel of the opposite parental line, the honeybee would not alight on the umbel and moved on to an umbel of the parental line it had been foraging on.

Honeybee movement between parental lines and anthophile interaction

Foraging honeybees that were followed from a male-fertile umbel seemed to prefer male-fertile above male-sterile umbels and were more likely to forage along male-fertile lines than to switch to male-sterile lines, with the highest percentage of hops occurring between male-fertile umbels (80.1%) (Table 5.4). The same pattern was found for honeybees that were followed from a male-sterile umbel, preferring male-sterile above male-fertile umbels and hopping between male-sterile umbels 84.6% of the time (Table 5.4). Thus, honeybees tended to forage on either male-fertile or male-sterile umbels, but not on both in the same foraging trip and did not switch between male-fertile and male-sterile lines. It was not determined whether specific individual honeybees would always prefer foraging on either male-fertile or male-sterile umbels during consecutive foraging trip.

Very few interactions between foraging honeybees and other insect visitors were recorded with only 12.3% and 5.9% of foraging honeybees encountering other insect visitors on male-fertile and male-sterile umbels, respectively (Table 5.5). Most of the interactions on the umbels involved other honeybees (Table 5.5). The rest of the encounters involved species of Diptera, non-*Apis* bees and Coccinellidae. Only four interactions (1.3% of the total number of interactions) on male-fertile umbels led to the honeybee hopping to a male-sterile umbel in the next four visits, while six interactions (2.7% of the total number of interactions) on male-sterile umbels led to the honeybee hopping from male-sterile to male-fertile umbels within the next four visits.

DISCUSSION

Onion nectar

The nectar volumes collected from the onion florets from my crop study sites were considerably lower than the mean nectar volumes of between 0.54 to 0.84 µl per floret per

day that were recorded by Hagler *et al*. (1990) in the U.S.A. However, Hagler *et al*., (1990) sampled nectar from umbels that were closed with paper bags for 24 hours prior to sampling, whereas here the umbels were more exposed to climatic variability by being closed with mesh bags for only a few hours before sampling. Therefore, the nectar volumes reported here would appear to be a more realistic representation of the nectar available to insect foragers under field conditions. The significantly cooler temperatures that were measured in the mesh bags as opposed to the measurements taken in mid-air are most likely attributable to the I-buttons in the mesh bags being less exposed to sunlight than those that were suspended in mid-air between the plants.

The significant differences in nectar volume detected between the parental lines and varieties are in accordance with the findings of other studies on hybrid seed crops. For example, Hagler *et al*. (1990) found significant differences in nectar volume secreted by five onion cultivars. In oilseed rape, nectar volumes differed significantly between seed quality genotypes and between male-sterile lines and their isogenic male-fertile counterparts (Pierre *et al*., 1999). Silva (1998) reported significant differences in the nectar volume and the consistency of production by nine hybrid onion parents and found that hybrid parents with higher nectar volumes and more consistent nectar production rates were more attractive to honeybees, suggesting compliance to the "optimal foraging theory" (Pyke *et al*., 1977; Smith, 1978). According to the nectar volumes reported here, honeybees would be more attracted to male-fertile lines with higher nectar volumes than to male-sterile lines with lower nectar volumes.

Nectar concentration has been found to vary between cultivars with concentrations ranging between 52-65% (Hagler *et al*., 1990), which is similar to the concentrations reported here. Lederhouse *et al*. (1968) found that sugar concentrations differed between hybrid onion parental lines and that nectar from male-fertile florets was more concentrated, which contrasts with the results reported for my study crops showing that, generally, male-sterile florets had more concentrated nectar. However, the difference in nectar concentrations between parental line umbels varied considerably from variety to variety. Silva (1998) also found significant differences in nectar concentration between hybrid onion parents but there was no significant relationship with honeybee visits.

Honeybees are generally attracted to sucrose solutions with 30-50% sugar concentrations (Waller, 1972). Sugar concentrations of 60% and above can cause bees to take more time

collecting the nectar as the nectar becomes too viscous for easy consumption (Roubik and Buchmann, 1984). This might add to the difference in attractiveness of hybrid onion parental lines, especially when the nectar of one parent is highly concentrated. However, when large numbers of honeybees are foraging, they tend to forage less selectively among sources with different sugar concentrations (Waller, 1972). This suggests that high stocking densities of managed hives may cause honeybees to forage less selectively among hybrid onion parental lines and is possibly one method of decreasing the generally discriminate nature of honeybee foraging behaviour, thus increasing movement of pollen between parental lines.

Consequently, higher nectar volumes produced by male-fertile florets (0.47 µl), and generally higher sugar concentrations of the nectar from male-sterile florets (> 50%) might be the determining factors causing honeybee visitation frequency to be higher on the male-fertile rows than on the male-sterile rows, as has been found in chapter 3. However, Waller *et al.* (1972) stated that merely considering nectar volume and concentration is insufficient to evaluate bee attractiveness to onion nectar, and suggest a more complete investigation of nectar quality and quantity, especially in terms of other nectar constituents apart from sugars.

Umbel scent

Silva (1998) identified 22 volatile compounds from onion flowers, of which only five were present in the data presented here. Silva (1998) also reported that sulphur-containing compounds were the most prominent (12 of the 22 compounds identified), with dipropyl disulphide being the most abundant compound identified, which contrasts with data reported for this study where benzenoids were the most prominent. Benzaldehyde was by far the most abundant compound from both parents and all the varieties, while other compounds had relatively low abundances. Benzenoids are compound classes commonly found in floral fragrance profiles (Dudareva *et al.*, 2004). However, volatile sulphur compounds are responsible for the characteristic pungent aroma of onions and other *Allium* species and are often the most abundant compounds isolated from onion bulbs and green tissue (Albrand *et al.*, 1980).

Emission rates of volatile compounds vary and are governed by their biosynthesis via chemical pathways and the rates of compound release (Dudareva *et al.*, 2004). The significant differences in the scent profiles of the hybrid onion parents and varieties were attributed to many small contributions from a large number of compounds. This suggests that, rather than the presence or absence of specific compounds, the relative concentrations of the compounds

emitted added to the statistical differences detected between the odorant mixtures. Similar results have been found for snapdragon flowers where honeybees were able to discriminate between the scent profiles of cultivars differing only in relative concentrations of eight volatile compounds (Wright *et al.*, 2005).

Reinhard *et al.* (2010) trained honeybees to complex artificial odorant mixtures and found that trained honeybees responded significantly to certain "key odorants" in the mixtures, and less to non-key odorants. The study concluded that whether a volatile was a "key-odorant" depended entirely on the mixture and not on molecular structure, volatility or learning efficiency. Whether honeybees recognized a compound as a key odorant also depended on whether the compound was highly concentrated in the mixture, though this was only pertinent when the test mixture was presented in low concentrations (Reinhard *et al.*, 2010). Pelz *et al.* (1997) reported that high concentrations of odorant supported stronger associations in being more salient, even though the honeybees were unable to distinguish two different concentrations of the same odorant as qualitatively different stimuli. Ribbands (1955) stated that honeybees can distinguish between mixtures of odours containing only slightly different amounts of the same scents.

Silva (1998) tested honeybees' response to particular scent compounds identified from onion flowers and found that neither benzaldehyde nor dipropyl disulphide were particularly attractive to honeybee foragers. However, conditioning bees to these two odours with sugar solutions significantly increased their attractiveness. Thus, the difference in odour between hybrid onion parents would be a strong foraging cue used by honeybees to associate the varying scent profiles with the most rewarding nectar source. My findings suggest that the difference in the volatile scent profiles of hybrid parental lines enable honeybees to discriminate between parental lines, which would be accentuated in the event where nectar resources differ between the parental lines. It would therefore be advantageous if isogenic parents could be selected in breeding to have similar characteristics in terms of floral volatiles and their emission rates, so as to minimise discrepancies which would encourage selective foraging by honeybees, thereby negatively impacting cross-pollination.

Honeybee movement between parental lines and anthophile interaction

Honeybees showed considerable discrimination between, and preference for, either male-fertile or male-sterile umbels when foraging. This is in accordance with findings of other authors (e.g. Lederhouse *et al.*, 1972; Waters, 1972). However, in a capture-mark-recapture

experiment, Gary *et al.*, (1977) recovered about half the metal tags from foraging honeybees that were tagged on one parental line, from magnetic traps set up on the opposite parental lines. This suggested that individual honeybees did not always forage on the same parental line during different foraging trips. Free and Williams (1972) found that considerable amounts of onion pollen were passively transferred between honeybees within the hive. These findings suggest that, although honeybees may practice discrimination between the parental lines during single foraging trips, they may still transfer onion pollen sufficiently within the field as a result of pollen transfer within the hive and different foraging patterns between trips.

The low numbers of interactions that occurred between honeybees and other insect foragers on the umbels, and the low numbers of switches honeybees made between parental line umbels after an interaction, indicate that it is unlikely that behavioural interactions between foraging honeybees and other insect visitors would have a significant effect on the foraging patterns of honeybees visiting hybrid onion umbels, at least for the study sites investigated in this study. Most of the interactions that occurred were between two foraging honeybees, while other insect visitors were involved to a much lesser extent. The visitation frequency that was recorded for non-*Apis* visitors, and especially non-*Apis* bees, was considerably lower than for honeybees (see Chapter 3), and thus the occurrence for such interactions is mathematically very small. Therefore, it is unlikely that behavioural interactions between honeybees and non-*Apis* flower visitors on hybrid onions would translate into increased pollen transfer from male-fertile to male-sterile umbels and ultimately into increased seed yields. The ecosystem service benefits in this regard, appear, at least for South African hybrid onions, to be of minimal value (however, see Greenleaf and Kremen, 2006).

CONCLUSION

The discriminating behaviour shown by honeybees when foraging on hybrid onion parental lines seems to be driven firstly by differences in the nectar resources offered by each parental line. The distinction between parental lines could be further reinforced by differences in umbel scent, as honeybees associate good nectar sources with particular odours and generally practice floral constancy in that they only forage on one food source per foraging trip. The strong correlation between honeybee visitation frequency and onion

hybrid seed yield, shown in chapter 4, suggests that honeybee visitation strongly affects pollination levels. But the tendency of honeybees to forage along parental lines with few crossings between the lines, limits pollen transfer. Thus, the mechanism of pollen transfer by honeybees remains somewhat unclear. Other studies have shown that onion pollen on the bodies of honeybees is transferred between workers within the hive and that individual workers do not select the same parental lines in consecutive foraging trips. A comprehensive research study should focus on measuring nectar and odour differences between parental lines while documenting the foraging patterns of marked individual honeybees on these lines during single and consecutive foraging trips, and assessing pollen loads on marked foragers.

REFERENCES

Albrand, M., Dubois, P., Etievant, P.X., Gelin, R., Tokarska, B., 1980. Identification of a new volatile compound in onion (*Allium cepa*) and leek (*Allium porum*): 3,4-Dimethyl-2,5-dioxo-2,5-dihydrothiophene. *Journal of Agricultural and Food Chemistry* 28, 1037–1038.

Ali, M., Dowker, B.D., Currah, L., Mumford, P.M., 1984. Floral biology and pollen viability of parental lines of onion hybrids. *Annals of Applied Biology* 104, 167–174.

Backhaus, W., 1993. Color vision and color choice behavior of the honey bee. *Apidologie* 24, 309–331.

Baker, H.G., 1977. Non-sugar chemical constituents of nectar. *Apidologie* 8, 349–356.

Baker, H.G., Baker, I., 1982. Chemical constituents of nectar in relation to pollination mechanisms and phylogeny, in: Nitecki, M.H. (Ed.), *Biochemical aspects of evolutionary biology*. University of Chicago Press, Chicago, pp. 131–171.

Benedek, P., 1977. Behaviour of honeybees (*Apis mellifera* L.) in relation to the pollination of onion (*Allium cepa* L.) inflorescences. *Zeitschrift fur Angewandte Entomologie* 82, 414–420.

Brewster, J.L., 2008. *Onions and other vegetable Alliums, 2nd Edition*. CAB International, Oxon.

Chittka, L., Thomson, J.D., 1999. Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* 86, 361–377.

Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User manual / tutorial*. PRIMER-E, Plymouth.

Clarke, K.R., Warwick, R.M., 1998. Quantifying structural redundancy in ecological communities. *Oecologia* 113, 278–289.

Corbet, S.A., 1978. Bee visits and the nectar of *Echium vulgare* L. and *Sinapis alba* L. *Ecological Entomology* 3, 25–37.

Degrandi-Hoffman, G., Watkins, J.C., 2000. The foraging activity of honey bees *Apis mellifera* and non-*Apis* bees on hybrid sunflowers (*Helianthus annuus*) and its influence on cross-pollination and seed set. *Journal of Apicultural Research* 39, 37–45.

Dowker, B.D., Currah, L., Horobin, J.F., Jackson, J.C., Faulkner, G.J., 1985. Seed production of an F_1 hybrid onion in polyethylene tunnels. *Journal of Horticultural Science* 60, 251–256.

Dudareva, N., Pichersky, E., Gershenzon, J., 2004. Biochemistry of plant volatiles. *Plant Physiology* 135, 1893–1902.

Free, J.B., 1969. Influence of the odour of a honey bee colony's food stores on the behaviour of its foragers. *Nature* 222, 778.

Free, J.B., 1993. *Insect pollination of crops*. Academic Press, London, UK.

Free, J.B., Williams, I.H., 1972. The transport of pollen on the body hairs of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus* spp. L.). *Journal of Applied Ecology* 9, 609–615.

Gary, N.E., Witherell, P.C., Lorenzen, K., Marston, J.M., 1977a. The interfield distribution of honey bees foraging on carrots, onions and safflower. *Environmental Entomology* 6, 637–640.

Gary, N.E., Witherell, P.C., Lorenzen, K., Marston, J.M., 1977b. Area fidelity and intra-field distribution of honey bees during the pollination of onions. *Environmental Entomology* 6, 303–310.

Gary, N.E., Witherell, P.C., Marston, J.M., 1972. Foraging range and distribution of honey bees used for carrot and onion pollination. *Environmental Entomology* 1, 71–78.

George, R.A.T., 1999. *Vegetable seed production, 2nd Edition*. CAB Interantional Publishing, New York.

Grant, V., 1950. The flower constancy of bees. *Botanical Review* 16, 379–398.

Greenleaf, S.S., Kremen, C., 2006. Wild bees enhance honey bees ' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences* 103, 13890–13895.

Hagler, J.R., 1990. Honey bee (*Apis mellifera* L) response to simulated onion nectars containing variable sugar and potassium concentrations. *Apidologie* 21, 115–121.

Hagler, J.R., Cohen, A.C., Loper, G.M., 1990. Production and composition of onion nectar and honey bee (Hymenoptera: Apidae) foraging activity in Arizona. *Environmental Entomology* 19, 327–331.

Howlett, B.G., Donovan, B.J., McCallum, J.A., Newstrom, L.E., Teulon, D.A.J., 2005. Between and within field variability of New Zealand indigenous flower visitors to onions. *New Zealand Plant Protection* 58, 213–218.

Joerges, J., Kuttner, A., Galizia, G., Menzel, R., 1997. Representations of odours and odour mixtures visualized in the honeybee brain. *Nature* 387, 285-288.

Johannsmeier, M.F., 2005. *Beeplants of the south-western Cape*. Plant Protection Research Institute, Handbook No. 17. Pretoria.

Jones, H.A., Clarke, A.E., 1943. Inheritance of male sterility in the onion and the production of hybrid seed. *Proceedings of the American Society for Horticultural Science* 43, 189–194.

Knudsen, J.T., Tollsten, L., Bergstrom, L.G., 1993. Floral scents - a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* 33, 253–280.

Kumar, J., Gupta, J.K., 1993. Nectar sugar production and honeybee foraging activity in 3 species of onion (*Allium species*). *Apidologie* 24, 391–396.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1968. Onion pollination in New York. *New York's Food and Life Sciences Bulletin* 1, 8–9.

Lederhouse, R.C., Caron, D.M., Morse, R.A., 1972. Distribution and behavior of honey bees on onion. *Environmental Entomology* 1, 127–129.

Mayer, D.F., Lunden, J.D., 2001. Honey bee management and wild bees for pollination of hybrid onion seed. *Acta Horticulturae* 561, 275–278.

McGregor, S.E., 1976. Common vegetables for seed and fruit, in: McGregor, S.E. (Ed.), *Insect pollination of cultivated crop plants*. United States Department of Agriculture, Washington.

Millar, J.G., Sims, J.J., 1998. Preperation, cleanup and preliminary fractionation of extracts, in: Millar, J.G., Haynes, K.F. (Eds.), *Methods in Chemical Ecology*. Kluwer Academic Publishers, Norwell, pp. 1–37.

Nicolson, S.W., Thornburg, R.W., 2007. Nectar Chemistry, in: Nicolson, S.W., Nepi, M, Pacini, E. (Eds.), *Nectaries and Nectar*. Springer, pp. 215–264.

Nye, W.P., Shasha'a, N.S., Campbell, W.F., Hamson, A.R., 1973. Insect pollination and seed set of onions (*Allium cepa* L.). *Utah Agricultural Experiment Station Research Report* No. 6, 15.

Parker, F.D., 1982. Efficiency of bees in pollinating onion flowers. *Journal of the Kansas Entomological Society* 55, 171–176.

Pathak, C.S., 2000. Hybrid seed production in onion. *Journal of New Seeds* 1, 89–108.

Pelz, C., Gerber, B., Menzel, R., 1997. Odorant intensity as a determinant for olfactory conditioning in honeybees: roles in discrimination, overshadowing and memory consolidation. *The Journal of Experimental Biology* 200, 837–847.

Percival, M.S., 1961. Types of nectar in angiosperms. *New Phytologist* 60, 235–281.

Percival, M.S., 1965. *Floral biology*. Pergamon Press, New York.

Pierre, J., Mesquida, J., Marileau, R., Pham-Delegue, M.H., Renard, M., 1999. Nectar secretion in winter oilseed rape, *Brassica napus* - quantitative and qualitative variability among 71 genotypes. *Plant Breeding* 118, 471–476.

Pyke, G.H., Pulliam, H.R., Charnov, E.L., 1977. Optimal foraging: a selective review of theory and tests. *The Quarterly Review of Biology* 52, 137–154.

R Development Core Team, 2011. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. (www.r-project.org)

Raguso, R.A., 2001. Floral scent, olfaction, and scent-driven foraging behavior, in: Chittka, L., Thompson, J.D. (Eds.), *Cognitive ecology of pollination; animal behavior and floral evolution*. Cambridge University Press, Cambridge, UK, pp. 83–105.

Reinhard, J., Sinclair, M., Srinivasan, M. V, Claudianos, C., 2010. Honeybees learn odour mixtures via a selection of key odorants. *PloS one* 5, e9110.

Ribbands, C.R., 1955. The scent perception of the honeybee. *Proceedings of the Royal Society B series* 143, 367–379.

Roubik, D.W., Buchmann, S.L., 1984. Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. *Oecologia* 61, 1–10.

Shanmugasundaram, S., 1998. *Onion cultivation and seed production*. Shanhua, Tainan, AVRDC.

Silva, E.M., 1998. *The effect of onion (*Allium cepa *L.) flower characteristics on its pollination by the honey bee (*Apis mellifera *L.)*. MSc thesis, Washington State University.

Silva, E.M., Dean, B.B., 2000. Effect of nectar composition and nectar concentration on honey bee (Hymenoptera: Apidae) visitations to hybrid onion flowers. *Journal of Economic Entomology* 93, 1216–1221.

Silva, E.M., Dean, B.B., Hiller, L., 2004. Patterns of floral nectar production of onion (*Allium cepa* L.) and the effect of environmental conditions. *Journal of the American Society for Horticultural Science* 129, 299–302.

Silva, E.M., Dean, B.B., Hiller, L.K., 2003. Honey Bee (Hymenoptera: Apidae) foraging in response to preconditioning with onion flower scent compounds. *Journal of Economic Entomology* 96, 1510–1513.

Smith, J.M., 1978. Optimization theory in evolution. *Annual Review of Ecology and Systematics* 9, 31–56.

Tholl, D., Rose, U.R.S., 2006. Detection and identification of floral scent compounds., in: Dudareva, N., Pichersky, E. (Eds.), *Biology of floral scent*. CRC Press, Taylor & Francis Group, New York, USA, pp. 3–35.

Waddington, K.D., 1980. Flight patterns of foraging bees relative to density of artificial flowers and distribution of nectar. *Oecologia* 44, 199–204.

Waller, G.D., 1972. Evaluating responses of honey bees to sugar solutions using an artificial-flower feeder. *Annals of the Entomological Society of America* 65, 857–862.

Waller, G.D., Carpenter, E.W., Ziehl, O.A., 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. *Journal of the American Society of Horticultural Science* 97, 535–539.

Waters, N.D., 1972. Honeybee activity in blooming onion fields in Idaho. *American Bee Journal* 112, 218–219.

Williams, I.H., Free, J.B., 1974. The pollination of onion (*Allium cepa* L.) to produce hybrid seed. *Journal of Applied Ecology* 11, 409–417.

Wills, A.B., North, C., 1978. Problems of hybrid seed production. *Acta Horticulturae* 83, 31–36.

Witter, S., Blochtein, B., 2003. Effect of pollination by bees and other insects on the production of onion seeds. *Pesquisa Agropecuária Brasileira* 38, 1399–1407.

Woyke, H.W., 1981. Some aspects of the role of the honeybee in onion seed production in Poland. *Acta Horticulturae* 111, 91–98.

Wright, G.A., Lutmerding, A., Dudareva, N., Smith, B.H., 2005. Intensity and the ratios of compounds in the scent of snapdragon flowers affect scent discrimination by honeybees (*Apis mellifera*). *Journal of Comparative Physiology* A 191, 105–14.

Table 5.1: Mean daily nectar volumes and sugar concentrations (± S.D) measured in onion florets (N) of between 7-10 male-fertile (MF) and 7-10 male-sterile (MS) umbels from 5 different hybrid onion varieties.

	Nectar Volume (µl)				Sugar Concentration (%)			
	N	MF	N	MS	N	MF	N	MS
Variety E	50	0.59 ± 0.24^{a}	49	0.38 ± 0.19^{b}	50	57.7 ± 5.8^{x}	44	64.0 ± 8.0^{y}
Variety F	73	0.48 ± 0.21^{a}	63	0.23 ± 0.14^{b}	73	54.2 ± 9.3^{x}	48	49.4 ± 9.2^{x}
Variety I	58	0.36 ± 0.16^{a}	56	0.26 ± 0.12^{b}	55	63.6 ± 5.3^{x}	55	60.1 ± 8.9^{x}
Variety P	60	0.39 ± 0.16^{a}	60	0.38 ± 0.18^{b}	45	52.5 ± 8.3^{x}	39	57.9 ± 10.7^{y}
Variety Q	60	0.55 ± 0.22^{a}	60	0.35 ± 0.16^{b}	52	49.0 ± 6.4^{x}	58	52.9 ± 6.1^{y}
Overall	301	0.47 ± 0.22^{a}	288	0.30 ± 0.17^{b}	276	55.4 ± 8.8^{x}	244	56.6 ± 9.9^{y}

Letters in superscript indicate significant differences between MF and MS umbels within each variety in terms of nectar volume (a, b) and sugar concentration (x, y). (Two-tailed *t*-tests, $P < 0.05$)

Table 5.2: Average relative amounts (%) of compounds emitted by male-fertile (MF) and male-sterile (MS) umbels of four different hybrid onion varieties during peak (>50%) blooming.

Compound	CAS	KRI	Variety E		Variety F – Farm 1		Variety F – Farm 2		Variety I	
			MF (n = 10)	MS (n = 8)	MF (n = 6)	MS (n = 6)	MF (n = 10)	MS (n = 8)	MF (n = 11)	MS (n = 10)
ALIPHATICS										
Unsaturated hydrocarbon										
(6*E*)-2,6-Dimethyl-2,6-octadiene	2792-39-4	1467	0.91 ± 0.21 (6)	-	-	0.06 ± 0.06 (1)	-	-	-	-
Aliphatic acids										
Acetic acid	64-19-7	1526	1.86 ± 0.56 (9)	0.30 ± 0.16 (3)	1.69 ± 1.28 (3)	0.33 ± 0.21 (3)	0.60 ± 0.41 (3)	0.11 ± 0.11 (1)	1.12 ± 0.35 (9)	2.55 ± 1.06 (9)
Aldehydes										
Hexanal	66-25-1	1161	2.33 ± 0.30 (10)	4.78 ± 1.90 (7)	4.75 ± 0.89 (6)	2.04 ± 0.69 (5)	5.74 ± 1.48 (10)	2.57 ± 0.50 (8)	1.93 ± 0.75 (11)	3.08 ± 0.78 (10)
Heptanal	111-71-7	1254	4.61 ± 1.54 (9)	2.73 ± 0.87 (5)	1.73 ± 1.50 (2)	4.04 ± 1.38 (5)	15.40 ± 9.26 (10)	3.40 ± 0.65 (8)	1.29 ± 0.33 (9)	3.33 ± 0.38 (10)
2-Hexenal	6728-26-3	1282	0.23 ± 0.15 (3)	0.43 ± 0.19 (5)	0.89 ± 1.41 (2)	0.12 ± 0.08 (3)	0.24 ± 0.21 (2)	0.03 ± 0.02 (2)	0.01 ± 0.00 (5)	0.11 ± 0.02 (9)
(*Z*)-2-Heptenal	57266-86-1	1390	2.08 ± 0.36 (10)	1.34 ± 0.33 (8)	0.79 ± 0.26 (4)	0.72 ± 0.25 (5)	1.87 ± 0.53 (10)	1.67 ± 0.50 (8)	0.69 ± 0.12 (11)	1.73 ± 0.23 (9)
(*E*)-2-Octenal	2548-87-0	1498	0.53 ± 0.10 (10)	0.21 ± 0.04 (7)	0.25 ± 0.10 (4)	0.25 ± 0.10 (4)	0.58 ± 0.25 (10)	0.64 ± 0.22 (8)	0.26 ± 0.07 (10)	0.60 ± 0.08 (10)
(2*E*,4*E*)-Hepta-2,4-dienal	04313-03-5	1565	-	0.06 ± 0.02 (6)	0.05 ± 0.04 (2)	0.01 ± 0.01 (1)	0.05 ± 0.04 (2)	0.02 ± 0.02 (1)	trace (1)	-
Ketones										
2-Undecanone	112-12-9	1673	0.44 ± 0.11 (10)	0.17 ± 0.10 (3)	6.28 ± 5.21 (5)	0.29 ± 0.13 (5)	0.78 ± 0.40 (5)	0.57 ± 0.34 (3)	3.74 ± 2.55 (10)	6.48 ± 3.41 (10)
4-Oxoisophorone	1125-21-9	1777	0.32 ± 0.05 (9)	0.18 ± 0.03 (7)	0.07 ± 0.03 (4)	0.12 ± 0.04 (6)	0.12 ± 0.05 (6)	0.17 ± 0.04 (8)	0.13 ± 0.02 (11)	0.16 ± 0.02 (10)
2-Tridecanone	593-08-8	1892	-	-	-	-	0.40 ± 0.27(4)	-	0.46 ± 0.31 (8)	0.46 ± 0.31 (8)
Alcohols										
1-Pentanol	71-41-0	1302	0.80 ± 0.42 (5)	0.92 ± 0.49 (4)	-	0.14 ± 0.14 (1)	1.88 ± 1.332 (2)	0.12 ± 0.12 (3)	1.04 ± 0.32 (9)	1.04 ± 0.32 (9)
1-Hexanol	111-27-3	1407	1.50 ± 0.64 (8)	0.58 ± 0.22 (8)	0.83 ± 0.37 (5)	0.63 ± 0.20 (6)	4.09 ± 1.86 (10)	1.83 ± 0.53 (8)	0.84 ± 0.22 (11)	4.17 ± 0.59 (10)
3-Hexen-1-ol	544-12-7	1439	0.11 ± 0.07 (3)	1.12 ± 0.33 (8)	0.16 ± 0.07 (5)	0.15 ± 0.06 (5)	0.73 ± 0.38 (10)	1.67 ± 1.55 (7)	0.06 ± 0.01 (10)	0.09 ± 0.02 (10)
1-Octen-3-ol	3391-86-4	1509	2.71 ± 0.46 (10)	1.79 ± 0.76 (8)	0.98 ± 0.35 (4)	0.63 ± 0.18 (6)	1.72 ± 0.56 (10)	2.48 ± 0.57 (8)	1.20 ± 0.17 (11)	4.41 ± 0.34 (10)
1-Heptanol	111-70-6	1513	0.40 ± 0.08 (10)	0.12 ± 0.04 (5)	0.14 ± 0.07 (3)	0.16 ± 0.06 (4)	0.30 ± 0.13 (6)	0.31 ± 0.08 (8)	0.15 ± 0.03 (11)	0.40 ± 0.10 (7)
1-Octanol	111-87-5	1621	3.27 ± 0.57 (10)	1.09 ± 0.17 (8)	1.96 ± 0.65 (5)	0.68 ± 0.43 (2)	1.61 ± 0.47 (10)	1.64 ± 0.45 (8)	1.87 ± 0.41 (11)	2.88 ± 0.40 (10)
(*Z*)-2-Octen-1-ol	18409-17-1	1683	0.31 ± 0.07 (9)	0.16 ± 0.04 (6)	0.17 ± 0.10 (3)	0.26 ± 0.10 (5)	0.61 ± 0.28 (8)	0.49 ± 0.19 (8)	0.21 ± 0.08 (7)	0.72 ± 0.10 (10)
1-Nonanol	143-08-8	1728	0.76 ± 0.15 (9)	-	0.62 ± 0.62 (1)	0.25 ± 0.11 (5)	0.57 ± 0.23 (8)	0.80 ± 0.29 (8)	0.63 ± 0.16 (11)	0.76 ± 0.15 (9)

Compounds are identified by common names and CAS (Chemical Abstracts Service) registry numbers and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentage of total peak area. The number of samples in which the compounds were identified is given in parentheses. The smallest possible subset of compounds reflecting the observed patterns is indicated in bold.

Table 5.2: continued.

Compound	CAS	KRI	Variety E		Variety F – Farm 1		Variety F – Farm 2		Variety I	
			MF (n = 10)	MS (n = 8)	MF (n = 6)	MS (n = 6)	MF (n = 10)	MS (n = 8)	MF (n = 11)	MS (n = 10)
BENZENOIDS										
Benzaldehyde	100-52-7	1598	57.32 ± 4.62 (10)	67.66 ± 3.47 (8)	57.75 ±10.38(6)	76.86 ± 3.93 (6)	44.39 ± 8.12 (10)	68.61 ± 6.79 (8)	67.96 ± 5.34 (11)	55.54 ± 2.93 (10)
Methyl benzoate	93-58-3	1704	5.53 ± 1.40 (10)	3.15 ± 0.63 (8)	0.52 ± 0.13 (5)	0.31 ± 0.09 (5)	1.61 ± 0.50 (10)	3.30 ± 1.95 (8)	3.10 ± 0.81 (11)	0.50 ± 0.11 (10)
Ethyl benzoate	93-89-0	1749	3.43 ± 1.09 (10)	0.45 ± 0.10 (7)	0.34 ± 0.13 (4)	0.27 ± 0.15 (5)	1.55 ± 0.45 (8)	1.30 ± 0.55 (8)	2.72 ± 1.22 (11)	0.43 ± 0.09 (10)
Propyl benzoate	2315-68-6	1846	0.65 ± 0.19 (9)	0.19 ± 0.05 (6)	0.07 ± 0.03 (3)	0.05 ± 0.02 (3)	0.18 ± 0.08 (7)	0.02 ± 0.55 (2)	0.21 ± 0.07 (9)	-
Benzyl alcohol	100-51-6	1960	4.73 ± 0.90 (10)	2.57 ± 0.64 (8)	2.25 ± 0.94 (5)	2.50 ± 0.90 (6)	6.38 ± 1.81 (10)	4.21 ± 1.16 (8)	5.96 ± 0.99 (11)	6.33 ± 0.97 (10)
Phenylethyl Alcohol	60-12-8	1997	0.42 ± 0.09 (10)	1.64 ± 1.12 (7)	0.12 ± 0.06 (3)	0.28 ± 0.11 (6)	0.94 ± 0.30 (9)	0.85 ± 0.34 (8)	1.28 ± 0.37 (11)	1.10 ± 0.20 (10)
Pentyl benzoate	2049-96-9	2064	-	-	-	-	-	-	0.07 ± 0.02 (7)	0.07 ± 0.02 (8)
Phenol	108-95-2	2089	0.46 ± 0.08 (10)	0.57 ± 0.07 (8)	0.40 ± 0.25 (5)	0.36 ± 0.08 (6)	0.31 ± 0.05 (10)	0.42 ± 0.08 (8)	0.27 ± 0.02 (11)	0.30 ± 0.03 (10)
Hexyl benzoate	6789-88-4	2171	-	-	-	-	-	-	0.04 ± 0.02 (3)	0.10 ± 0.04 (5)
Acetylanisole	100-06-1	2238	0.92 ± 0.24 (9)	0.07 ± 0.03 (6)	0.04 ± 0.02 (3)	0.11 ± 0.07 (5)	0.26 ± 0.13 (8)	0.26 ± 0.17 (8)	0.65 ± 0.13 (10)	0.65 ± 0.13 (10)
Veratraldehyde	120-14-9	2479	0.04 ± 0.02 (5)	0.02 ± 0.01 (4)	-	0.01 ± 0.01 (3)	0.07 ± 0.06 (5)	0.01 ± 0.01 (3)	0.06 ± 0.02 (10)	0.06 ± 0.02 (10)
Benzyl Benzoate	120-51-4	2751	0.19 ± 0.10 (8)	0.01 ± 0.01 (2)	-	0.10 ± 0.10 (1)	0.03 ± 0.02 (4)	0.06 ± 0.05 (6)	0.35 ± 011 (8)	0.25 ± 0.07 (10)
LACTONES										
5-Ethyldihydro-2(3H)-furanone	695-06-7	1786	-	0.04 ± 0.02 (4)	-	0.01 ± 0.01 (2)	0.01 ± 0.01 (1)	0.06 ± 0.02 (6)	-	-
SULPHUR-CONTAINING COMPOUNDS										
Methyl propyl disulfide	2179-60-4	1304	-	-	0.99 ± 0.39 (4)	0.63 ± 0.27 (4)	1.35 ± 0.84 (5)	0.27 ± 0.14 (3)	-	-
3,4-Dimethylthiophene	632-15-5	1321	0.91 ± 0.35 (8)	1.12 ± 0.90 (6)	6.08 ± 4.47 (5)	3.14 ± 1.74 (6)	0.74 ± 0.18 (9)	0.85 ± 0.38 (5)	0.30 ± 0.09 (7)	0.06 ± 0.01 (7)
1,3-Dithiane	505-23-7	1355	-	-	2.20 ±2.01 (3)	0.10 ± 0.10 (1)	-	-	0.04 ± 0.04 (1)	trace (4)
Dipropyl disulphide	629-19-6	1449	1.60 ± 0.37 (10)	6.02 ± 2.27 (8)	4.71 ± 1.70 (6)	3.45 ± 2.06 (5)	4.29 ± 1.92 (10)	0.67 ± 0.21 (8)	0.07 ± 0.01 (11)	0.51 ± 0.08 (10)
Dimethyl trisulphide	3658-80-8	1454	0.02 ± 0.01 (2)	-	0.59 ± 0.29 (4)	0.11 ± 0.06 (4)	0.12 ± 0.06 (3)	0.31 ± 0.13 (6)	0.19 ± 0.07 (11)	0.09 ± 0.06 (3)
1,2-Dithiolane	557-22-2	1511	-	0.78 ± 0.36 (5)	1.49 ± 1.07 (4)	0.57 ± 0.38 (2)	0.21 ± 0.14 (2)	-	-	-
Dipropyl trispulfide	6028-61-1	1759	-	0.08 ± 0.05 (3)	0.05 ± 0.05 (1)	0.14 ± 0.09 (4)	-	-	-	-
trans-3,5-Diethyl-1,2,4-trithiolane	54644-28-9	1894	-	0.08 ± 0.05 (4)	0.04 ± 0.03 (2)	0.07 ± 0.04 (5)	0.07 ± 0.04 (3)	0.12 ± 0.07 (5)	-	-
UNKNOWN										
Unknown		2033	0.61 ± 0.46 (9)	0.03 ± 0.01 (5)	0.07 ± 0.03 (3)	0.18 ± 0.10 (5)	0.13 ± 0.05 (7)	0.16 ± 0.06 (8)	1.08 ± 0.38 (10)	0.27 ± 0.06 (10)

Table 5.3: Volatile scent compounds that contributed to within-group similarities with a cumulative contribution of ≤ 50%. The numbers of compounds contributing up to 100% within-group similarity are also shown, together with the average within-group similarities.

Compound	Variety E	Variety F*	Variety F*	Variety I	MF	MS
	n = 18	n = 12	n = 18	n = 21	n = 37	n = 32
Benzaldehyde	30.63%	42.75%	31.14%	28.99%	29.70%	33.26%
Benzyl alcohol	6.20%		6.83%	7.76%	7.37%	6.21%
1-Octen-3-ol				5.25%		5.07%
1-Octanol				4.57%		
1-Hexanol				4.28%		
Methyl benzoate	5.84%				4.84%	
Hexanal	5.14%	7.09%	6.52%		5.53%	4.96%
Dipropyl disulphide	5.13%	5.35%				
Heptanal			8.26%		4.88%	4.85%
Cumulative Total	*52.93%*	*55.2%*	*52.76%*	*50.84%*	*52.33%*	*54.36%*
Nr. of compounds contributing to 100% similarity within groups	36	35	40	32	39	39
Average within-group similarity	74.07%	63.17%	61.46%	77.96%	68.50%	74.35%

* Indicates the same variety that was grown on two different farms with no significant difference in compound composition (R = 0.13, P = 6.1%), MF = male-fertile flower heads, MS = male-sterile flower heads

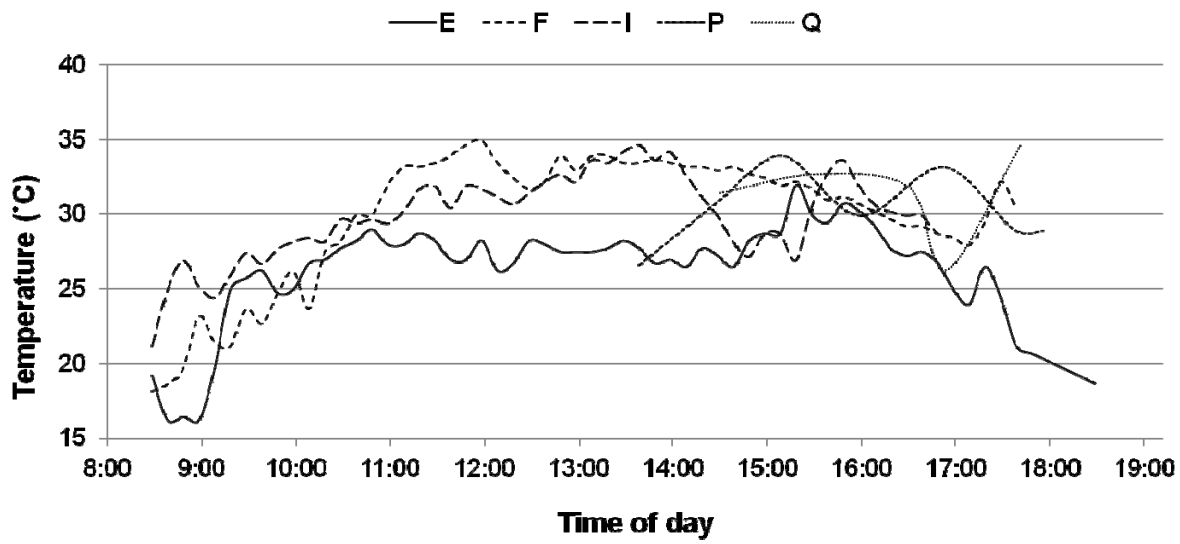
Table 5.4: The movement of honeybees along, and between parental lines, when followed along male-fertile (MF) and male-sterile (MS) lines on 7 hybrid onion varieties. The percentages of occurrences are indicated in brackets.

Parent	Nr. of honeybees (N)	Honeybee movement along and between parental lines								χ_3^2	P
		MF – MF		MF – MS		MS – MS		MS - MF			
MF	262	1079	(80.1)	100	(7.4)	125	(9.3)	43	(3.2)	2191.87	< 0.001
MS	218	77	(7.1)	29	(2.7)	920	(84.6)	61	(5.6)	2066.23	< 0.001

Table 5.5: The total numbers of interactions recorded between foraging honeybees and other flower-visiting insects on male-fertile (MF) and male-sterile (MS) umbels. The percentage of occurrences is indicated in brackets.

Parent	Nr. of foraging bouts (N)	Flower-visitors				Total nr. of interactions
		Honeybees	Diptera	non-*Apis* bees	Coccinellidae	
MF	318	15 (4.7)	12 (3.8)	4 (1.3)	8 (2.5)	39 (12.3)
MS	222	7 (3.2)	0	0	6 (2.7)	13 (5.9)

a



b

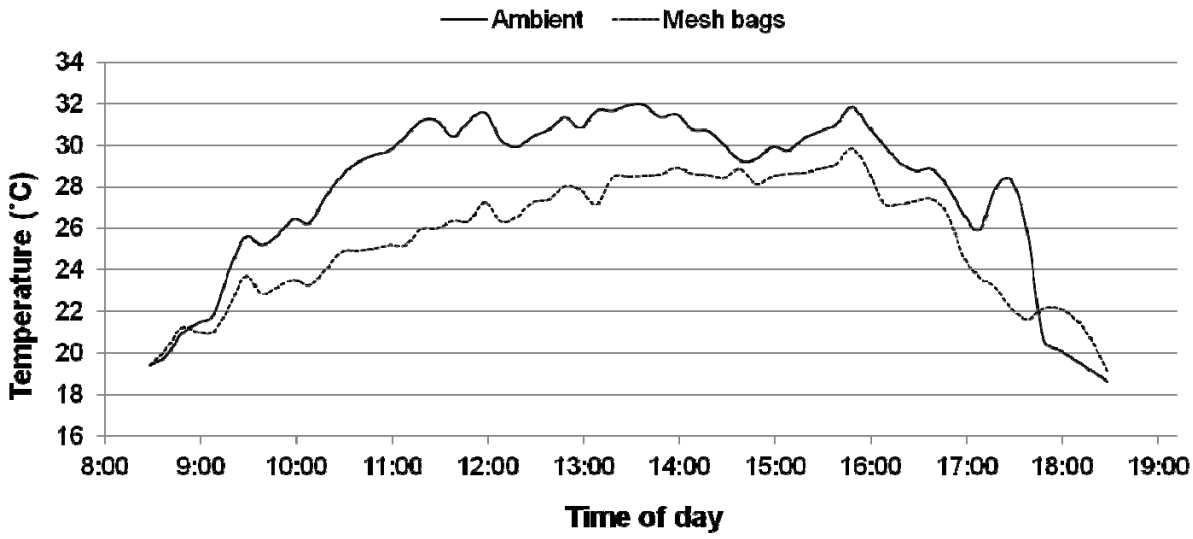


Figure 5.1: a) Ambient temperatures (˚C) recorded during nectar sampling days (varieties E, F & I) and nectar sampling periods (varieties P & Q). b) Mean ambient and mesh bag temperatures recorded during nectar sampling days (varieties E, F & I).

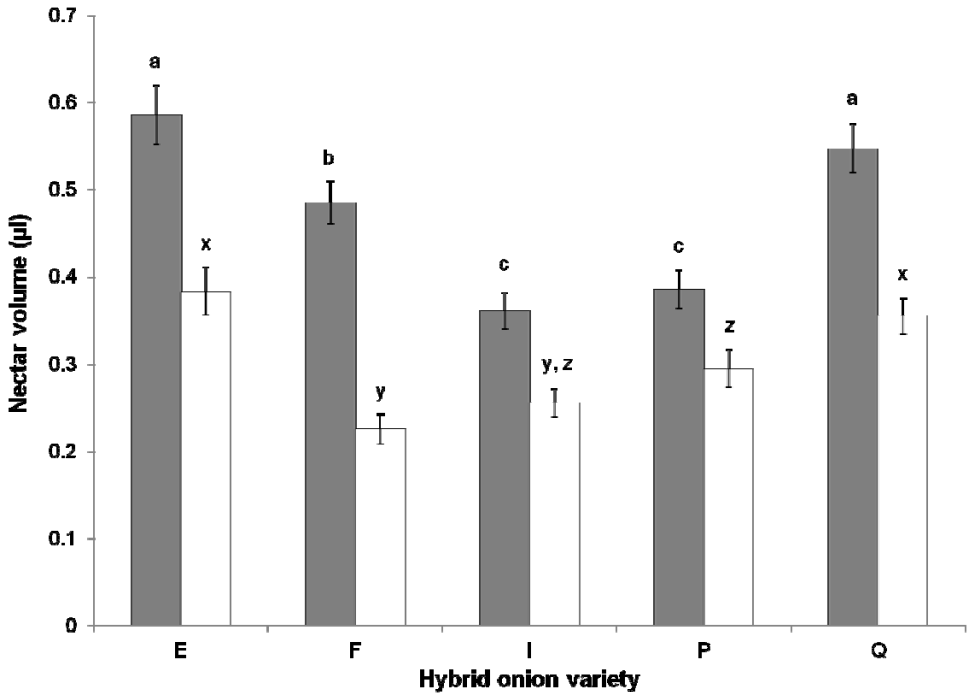


Figure 5.2: Mean (± S.E.) nectar volumes (µl) recorded from florets of male-fertile (grey bars) and male-sterile (white bars) hybrid onion umbels. The letters indicate significant differences between the varieties within MF (a, b, c) and MS (x, y, z) umbels (two-tailed *t*-tests). Significant differences occurred between MF and MS umbels within all the varieties (see text for detail of statistical results).

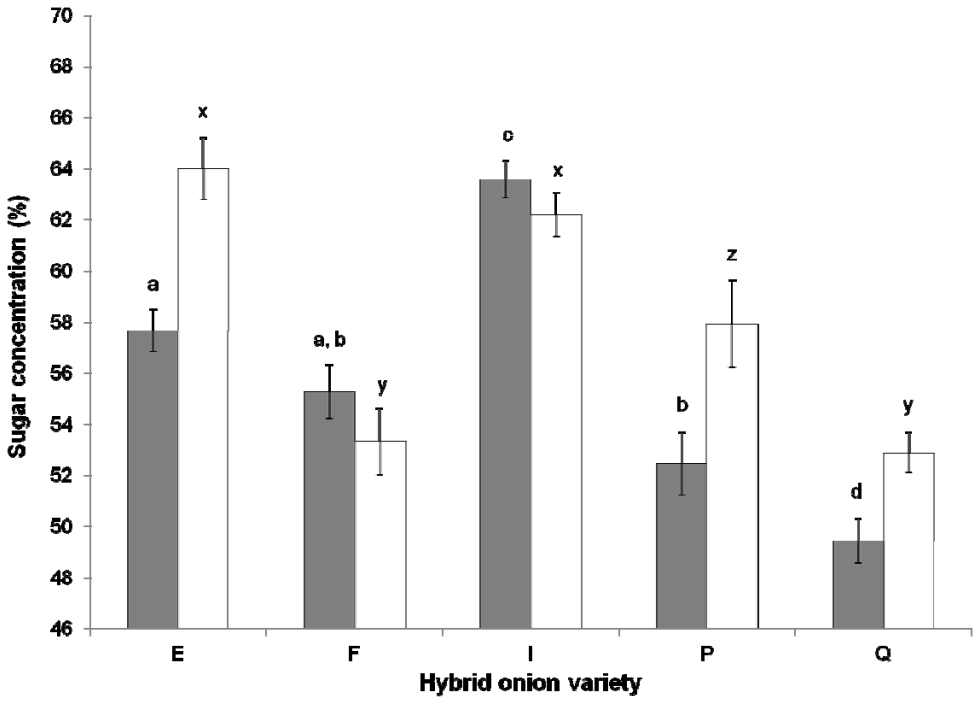


Figure 5.3: Mean (± S.E.) sugar concentrations (%) recorded from florets of male-fertile (grey bars) and male-sterile (white bars) hybrid onion umbels. The letters indicate significant differences between the varieties within MF (a-d) and MS (x-z) umbels (two-tailed *t*-tests). Significant differences occurred between MF and MS umbels within varieties E, P and Q (see text for detail of statistical results).

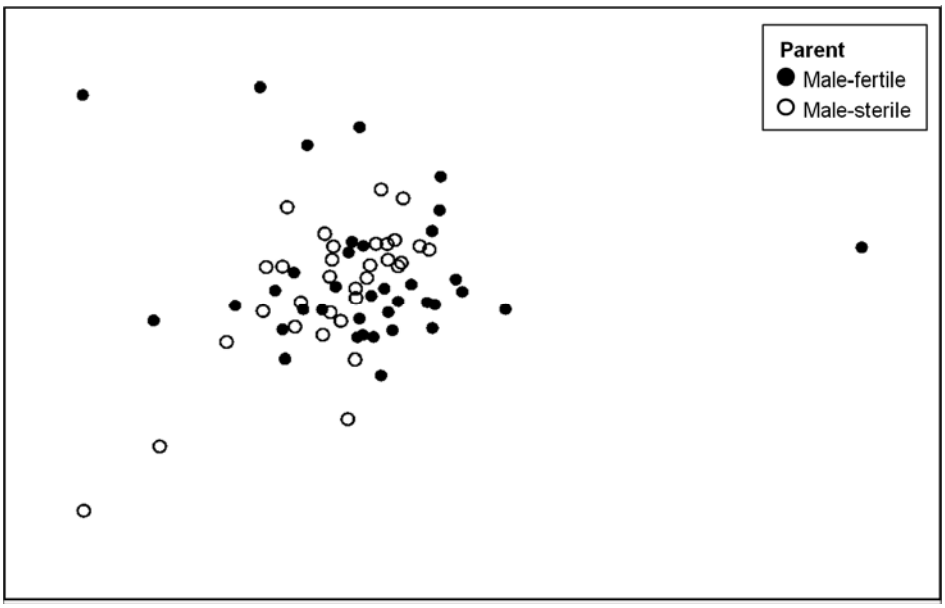


Figure 5.4: Non-metric multidimensional scaling (MDS), based on Bray-Curtis similarities of the odour composition of male-fertile and male-sterile hybrid onion umbels comprising 41 compounds from 69 male-fertile and male-sterile onion flower heads (umbels). (2D stress value = 0.16).

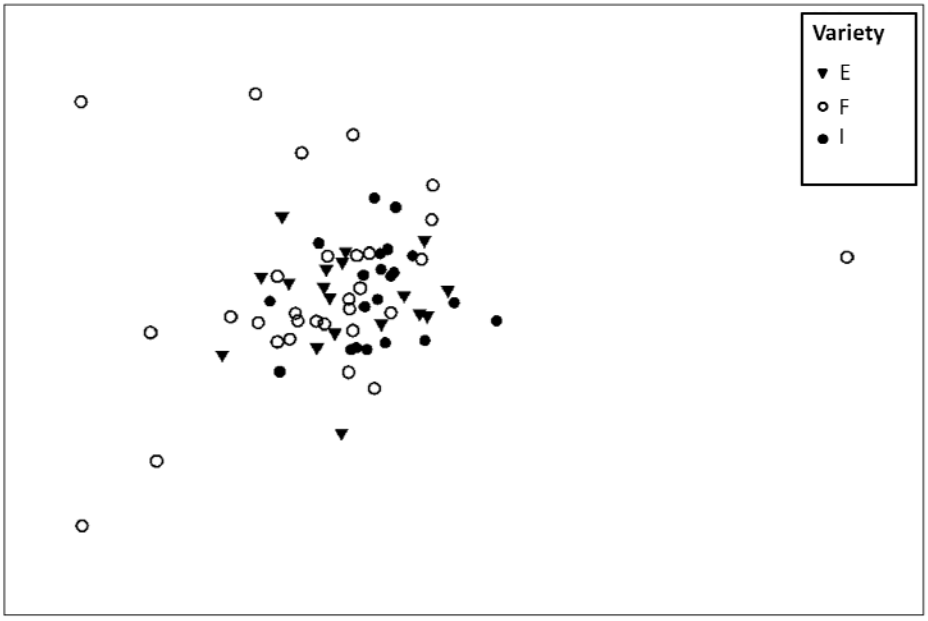


Figure 5.5: Non-metric multidimensional scaling (MDS), based on Bray-Curtis similarities of the odour composition of the umbels of four different varieties (E, F & I) comprising 41 compounds from 69 male-fertile and male-sterile onion flower heads (umbels) (2D stress value = 0.16).

6

GENERAL DISCUSSION

Animal pollination is a critical ecosystem service that regulates the reproduction of most angiosperm plants (Millenium Ecosystem Assessment, 2005). Furthermore, pollinated agricultural crops vary in dependence on animal pollination for production, from crops that benefit from pollinator visitation to crops that are completely dependent on pollination for successful yields (Klein *et al.*, 2007). The production of F_1 hybrid seed is completely dependent on insect pollination; for example, the bee factor for onion hybrid seed production is an estimated 95% (Johannsmeier, 2005; Brewster, 2008). Although the honeybee is still the species most widely used to pollinate agricultural crops, native, wild pollinator communities may deliver considerable pollination ecosystem services that may lower the cost of production, but this free service is often unrecognised by farmers (Kremen *et al.*, 2002, 2004). Several authors have linked good quality adjacent source habitats and subsequent pollinator diversity in crop fields to significant increases in crop yields (Carvalheiro *et al.*, 2010; Greenleaf & Kremen, 2006; Klein *et al.*, 2003; Kremen *et al.*, 2002; Olschewski *et al.*, 2006; Richards, 2001; Ricketts *et al.*, 2004; Steffan-dewenter & Tscharntke, 1999). The research presented in this thesis aimed to determine whether onion hybrid seed growers in South Africa benefit from pollination ecosystem services delivered by wild pollinator communities and if anthophile diversity within the crops was supported by the availability of natural habitat near the crops. If this is the case, it would be in the farmers' interest to manage local natural habitats in ways that would allow them to secure, and take advantage of this free service (Morandin and Winston, 2006; Morandin *et al.*, 2007). Wild pollinator communities could also provide a form of protection against potential losses of honeybees (Winfree *et al.*, 2007).

Vegetable seed is one of the main agricultural products produced in the Klein Karoo and southern Karoo regions of the Western Cape, South Africa, and contributes significantly to the local economy. The market value of onion seed produced in South Africa for the 2010/2011 production year was estimated at R 117.99 million with the production of 116,138 kg onion hybrid seed and 389,911 kg open pollinated onion seed during the same period (www.sansor.org). Many local farmers in the region produce vegetable seed together with a variety of other farming produce, including ostrich products for which the region is well

known (Beyleveld, 1967), in an effort to spread the risk of market exposure because farming in South Africa is not subsidised.

The usual stocking density used to pollinate onion hybrid seed crops grown in South Africa is approximately 10 honeybee hives per hectare. Managed honeybee hives in the Western Cape are occupied by colonies of the indigenous and endemic Cape honeybee (*Apis mellifera capensis* Esch) and hives are commonly stocked by trapping feral swarms on the move (McGregor, 1976; Hepburn, 1993; Hepburn and Radloff, 1998; Johannsmeier, 2001). However, some farmers make considerable use of wild honeybees that visit their crops, and recognise this as a valuable and cost-effective pollination ecosystem service. These farmers usually use lower stocking densities of managed hives and deploy more managed honeybee colonies only when needed. Some farmers even provide non-conventional, artificial nesting sites close to the crops for wild honeybee colonies to move into. These colonies are not regarded as managed because honey is not harvested from them.

The majority of managed honeybee hives that are used for onion hybrid seed pollination in the Klein Karoo are permanently resident on the farms and are owned and managed by local beekeepers. These resident hives are locally relocated to desired positions during crop flowering periods for optimal pollination, and managed hives are only occasionally sourced from outside the Klein Karoo for pollination purposes. This custom local practice was reinforced with the recent discovery of the highly infectious American Foul Brood disease in managed colonies during the first quarter of 2009, in an effort to prevent the disease from spreading (Allsopp, 2009; Steyn, 2009). Because of the more arid conditions prevalent in the southern Karoo which is unable to sustain large numbers of honeybees throughout the year, managed honeybee colonies are mostly externally sourced from other regions for pollination purposes in this region of onion hybrid seed production.

The Succulent Karoo biome that encompasses the two onion hybrid seed production regions has been declared a global biodiversity hotspot (Myers *et al.*, 2000) and is renowned for its high plant diversity and magnificent spring flower displays. The pollinator community of the Succulent Karoo proved to be equally diverse (Hesse, 1938; Whitehead, 1984; Struck, 1994; Eardley, 1996; Gess, 1996, 2001; Manning and Goldblatt, 1996; Picker and Midgley, 1996; Kuhlmann, 2009). Coloured pan-trap sampling within 18 onion hybrid seed crops showed that a diversity of potential anthophile insects were present within the crops during flowering. Hymenoptera, Diptera and Coleoptera was well represented in the traps with

fewer Hemiptera and Lepidoptera species present. The Cape honeybee was the single most abundant species present in the traps.

However, the diversity of potential flower-visiting insects collected within the crops was not dependent on the percentage of natural habitat that surrounded the crop fields. The proximity of natural or semi-natural habitat is generally relatively close to cultivated crops in semi-arid regions, because crops are mostly cultivated in alluvial terraces along water courses that provide access to richer soils and irrigation water (Dean and Milton, 1995; Gess, 2001; Thompson *et al.*, 2005). Some bee and aculeate wasp species would be abundant along water courses because of the availability of water for nest building purposes (Gess, 2001). In addition, the farms in the Klein Karoo are relatively small and allow only for small-scale intensive cultivation within a matrix of non-arable land surfaces which is used generally for livestock grazing (J. Bekker, pers. comm).

The blooming onion crops could have offered essential floral resources that attracted a diversity of flower-visiting insects, regardless of whether the crops were situated in an agricultural matrix or in more natural landscapes. Westphal *et al.* (2003) found that the densities of bumble bees were determined by the availability of highly rewarding mass flowering crops, rather than the proportion of semi-natural habitats in agricultural landscapes. On the other hand, Diekötter *et al.* (2010) warn of potential disturbances of plant-pollinator interactions whereby mass-flowering crops provide abundant resources for certain functional trait groups, thereby disrupting resource-partitioning as a mechanism of co-existence for competitors.

However, less than 20% of the insect diversity sampled in the pan-traps were observed as visitors on the onion flowers. Many species that were sampled with the pan-traps are not primary nectar or pollen feeders, and can only be regarded as occasional flower visitors. Therefore, the diverse assemblages may have been caused by other factors rather than the abundance of onion floral resources. The two management practices that were measured during the study, crop health and irrigation method, significantly altered the insect assemblages within the crops. Crops with sub-standard health were characterized by unhealthy plants and were often overgrown with weed species that could offer alternative floral resources during onion flowering periods. Unhealthy crops seemed to host more diverse insect assemblages consisting mostly of numerous true fly species that could have been attracted to diseased and rotting onion plants. The proximity of ostrich camps within

the more cultivated areas could also encourage a greater diversity of carrion fly species present within the hybrid onion crops. Methods used for irrigation purposes may determine the availability of water to various insect species, thereby possibly determining insect assemblages found within crops (Brown *et al.*, 1977; Gess, 2001; Mayer and Lunden, 2001).

Tuell and Isaacs (2009) sampled 40 species of bees with elevated coloured pan-traps in highbush blueberry crops (*Vaccinium corymbosum*) of which only 17 species were known to forage on the crop. Similarly, 44 bee species were sampled with the pan-traps in the onion hybrid seed crops of which 19 species were represented by only one or two individuals and could be regarded as rare species. However, only the honeybee, and rarely non-*Apis* bees, were observed visiting the hybrid onion flowers.

Honeybees were the most important insect visitors based on high visitation frequency and significant loads of onion pollen carried on their bodies. Very low visitation frequency rendered other insect visitors, mostly ladybird beetles, milkweed bugs and true flies, insignificant as potential onion flower pollinators, even though comparable onion pollen loads were present on at least one of the visitors, *Spilostethus pandurus* Scopoli (Hemiptera: Lygaeidae). Some authors have found non-*Apis* insect visitors to be efficient in pollinating onion flowers (Parker, 1982; Currah and Ockendon, 1983, 1984; Wilkaniec *et al.*, 2004). However, honeybees are often the most abundant visitors around, which is critical in attaining adequate pollination levels throughout the entire flowering period of the onion crops (Williams and Free, 1974; Caron et al., 1975; Benedek, 1977; Free, 1993). Two separate studies ranked the honeybee as the most important pollinator of onion hybrid seed crops in America, based on their abundance and efficiency (measured by body size, hairiness, and activity patterns) (Bohart *et al.*, 1970; Caron *et al.*, 1975).

Knowing the proportion of honeybee foragers that came from managed hives, and those that came from wild colonies resident in natural habitat near the crops, could give an indication of the degree of pollination ecosystem services received by the farmers. However, distinguishing between managed and wild honeybees in the field is very difficult. In addition, there was no significant relationship between honeybee visitation frequency and either managed hive density, or percentage natural habitat. Instead, honeybee visitation was significantly related to total annual rainfall, signalling at least the potential of an indirect ecosystem service through the provision of wild flower resources to sustain wild, as well as

resident, managed honeybee colonies, although other secondary factors that may result from rainfall variability also might have played a role.

The diversity of insects hand-sampled from the umbels increased as honeybee visitation increased, indicating similarities in the size of honeybee and non-*Apis* populations foraging on the onion flowers. This might be due to the equal attractiveness of hybrid onion varieties to honeybees and non-*Apis* visitors alike, or to the size of the insect populations present during the hybrid onion blooming season that could visit the onion flowers. Annual variability in rainfall may lead to secondary factors that could determine insect visitation to hybrid onion flowers; for example, increasing abundance of floral resources supporting pollinator population growth. Climates of arid and semi-arid regions are usually characterized by extreme temperatures and great variability in both the timing and amount of rainfall (Cowling *et al.*, 1986). For that reason, succulent Karoo vegetation responds well to rain and the flowering time of wild plants are governed by the timing of rainfall events to a large extent (Vlok and Schutte-Vlok, 2010). Early rains would lead to early flowering, but late rains would cause the veldt to flower later, which may coincide with the flowering of the onion hybrid seed crops that usually stretches between about late October until late November. Natural vegetation in flower during this time may compete with the blooming onion crops in attracting insect flower-visitors. This is a significant factor since hybrid onion are known to be generally unattractive to honeybees (Waller *et al.*, 1972; Hagler, 1990; Hagler *et al.*, 1990).

Rainfall variability may also lead to other secondary factors like soil moisture and water quality which may have direct effects on seed yield (e.g. Hawthorn, 1951). For example, potassium concentrations in the nectar of onion flowers have been linked with soil-salinity and these higher potassium concentrations appear to influence the attractiveness of the nectar for honeybees (Waller *et al.*, 1972). And, although it is unlikely that low rainfall would cause water stress in onion plants, since onion crops are irrigated according to very specific irrigation schedules (F. van der Merwe, pers. comm.), the water sourced from large dams that is used to irrigate the crops may be of lower quality because of evaporation, causing higher salt concentrations, which may deter honeybees from foraging on the onion flowers when sprinkle irrigation is used, or when this water causes increased soil-salinity (P. Burger, pers. comm.). Farmers may thus have perfect potential honeybee-delivered pollination services, but other factors can impede visitation, leading to degraded pollination services. Further research should test the effects of secondary factors caused by the variability in rainfall events on honeybee visitation frequency.

Honeybee visitation improved onion hybrid seed yield significantly, while neither non-*Apis* visitation frequency, nor the general diversity of anthophiles present within the crops had a significant effect on seed yield. The latter was expected, since the abundance and richness of anthophiles sampled with pan-traps was not observed on the onion flowers. The importance of managed vs. wild honeybees in bringing about increased seed yield remained unclear because of the lack of any significant relationship between seed yield and managed hive density or percentage natural habitat. The fact that neither seed yield, nor honeybee visitation frequency were related to managed hive density or the availability of natural habitat, indicates that other factors, which may include the attractiveness of the different hybrid onion varieties and secondary factors caused by total annual rainfall, were probably more important in determining the size of the foraging population of honeybees and non-*Apis* visitors on the blooming onion hybrid seed crops, and subsequent seed set resulting from insect visitation.

There was a marked difference in seed set between the two major production regions, with higher seed set attained in the southern Karoo compared to the Klein Karoo. There are often variation in hybrid seed yields produced in different production areas, often because of dissimilar physiological attributes like climate and soil type (Rai and Rai, 2006). Onion hybrid seed crops in the southern Karoo would be stronger and healthier due to the drier climate that is less favourable to onion pathogens (Cowling, 1986; Dean and Milton, 1995). Furthermore, the greater scarceness of wild flower resources in the southern Karoo, because of the drier climate, would force insects to forage on the blooming crops. Indigenous flower resources is important for the sustenance and growth of pollinator populations during early spring but ideally the natural veldt should offer no alternative floral resources during the flowering of the onion hybrid seed crops. Due to the more arid conditions prevalent in the southern Karoo, rented managed honeybee colonies are important in attaining pollination services in this production region and are primarily imported from other regions to pollinate the flowering crops.

South African seed companies reported that record vegetable seed yields were attained from the 2011 pollination season. This also happened to be a year of impressive and abundant wild flower displays after timely and copious rainfall throughout the production region (pers. obs.). However, many farmers experienced total losses of their onion hybrid seed crops the following year, solely because honeybees did not visit the onion flowers for reasons that are still very unclear and speculative (Le Roux, 2013). The phenomenon of

sporadic widespread crop failure in the production of onion hybrid seed has been a problem for decades (Waller *et al.*, 1972). Several factors have been suggested for causing occasional impaired yields, of which the most important is thought to be the unattractiveness of onion flowers to honeybees in comparison with other floral resources (Gary *et al.*, 1972, 1977), which is possibly due to a high potassium concentration in the nectar leading to insufficient honeybee visitation (Waller *et al.*, 1972; Hagler *et al.*, 1990).

In an effort to determine honeybee behaviour and foraging patterns on the onion flowers, foraging honeybees were followed and showed a noticeable preference for male-fertile lines of the onion hybrid seed crops investigated here, which is similar to others' findings (Williams and Free, 1974; McGregor, 1976; Woyke, 1981; Parker, 1982; Silva, 1998; Mayer and Lunden, 2001). Nevertheless, the population of foraging honeybees on the male-sterile flowers were still two thirds of the population of honeybee foragers on the male-fertile umbels. Male-fertile flowers offered larger volumes of less concentrated nectar which probably attracted more honeybees than the male-sterile lines. Honeybees foraged along parental lines rather than moving across lines, while very few inter-species interactions were recorded that caused honeybees to switch between parental lines, mainly because other foragers were so scarce, that the presence of non-*Apis* visitors did not amount to a viable pollination ecosystem service.

The significant difference in onion flower scent profiles between the parental lines could be a strong foraging cue used by honeybees to associate scent with the most rewarding nectar source, encouraging selective foraging through floral constancy. When honeybees test floral scent by hovering in front of the flowers of the opposite parent, it restricts opportunities for inadvertent contact with the floral reproductive parts, thereby limiting pollination. Hybrid onion breeding programs should attempt to take these parental line differences into account when selecting for favourable production traits. The selection for more similar traits might lessen honeybee discrimination between parental lines (Wills and North, 1978).

In conclusion, my results show that the successful production of onion hybrid seed in South Africa is totally dependent on honeybee visitation, and that considerable pollination ecosystem service is derived from wild honeybee colonies where managed hive stocking densities are low. In fact, honeybees were the only significant wild pollinator of onion flowers, based on their abundance and onion pollen loads. In addition, interactions between

foraging honeybees and non-*Apis* onion flower-visitors were unimportant in affecting the efficiency of honeybee pollination.

There is close interaction between onion hybrid seed pollination and the ecosystems of the two production regions with a fair degree of interdependence. Hybrid onion pollination in the Klein Karoo benefit markedly from sufficient and timely rainfall events that initiate abundant wild flower displays before the blooming of the onion hybrid seed crops, supporting the growth and maintenance of both resident managed and wild honeybee colonies. Large populations of honeybee foragers have been shown to forage more readily on less attractive floral resources (Waller, 1974) while honeybee visitation depend on colony population size (Waller *et al*., 1985). However, the flowering time of the natural veldt may prove to be a potential crucial factor that may disrupt successful hybrid onion pollination. On the other hand, onion flowers may provide copious floral resources for diverse communities of non-*Apis* flower-visitors after the natural veldt has finished flowering, although these visitors were not abundant on the onion flowers. In regions with fewer floral resources to sustain large wild honeybee populations, rented managed honeybee colonies imported from other regions is an important pollination service available to onion hybrid seed farmers.

Incomplete and variable pollination reduce average yield and yield stability, even more so for crops with high pollinator dependence, while declining yield growth with increased inputs may lead to the conversion of more land to cultivation (Garibaldi *et al*., 2011). Onion hybrid seed is a specialist crop that occupy relatively little ground and generally unit expansion is of low priority (Richards, 2001). Pollinator-dependent crops are on average five times more valuable per ton than crops not dependent on animal pollination (Gallai *et al.,* 2009). Although the cost of producing onion hybrid seed is comparatively high, with a relatively higher risk of crop failure; for example due to inadequate pollination, it remains a highly profitable crop that is worth the initial input costs (Wills and North, 1978). South African farmers can mostly gain satisfactory benefits from well managed natural habitats near their crops within the succulent Karoo landscape that has the potential to support large populations of wild honeybees that can offer abundant pollination ecosystem services during favourable climatic conditions. Nevertheless, managed honeybee colonies within the Western Cape region are invaluable to onion hybrid seed pollination where the landscape is not able to support large wild honeybee populations, or where additional honeybees are needed to increase the number of worker bees on the crops.

REFERENCES

Allsopp, M.H., 2009. *Bee disease threatens South African apiculture*. Plant Protection News, N79. Agricultural Research Council: Plant Protection Research Institute.

Benedek, P., 1977. Behaviour of honeybees (*Apis mellifera* L.) in relation to the pollination of onion (*Allium cepa* L.) inflorescences. *Zeitschrift fur Angewandte Entomologie* 82, 414–420.

Beyleveld, A.J., 1967. Ostrich Farming in South Africa. *Agrekon* 6, 14–16.

Bohart, G.E., Nye, W.P., Hawthorn, L.R., 1970. *Onion pollination as affected by different levels of pollinator activity*. Utah Agricultural Experiment Station Bulletin No. 482.

Brewster, J.L., 2008. *Onions and other vegetable Alliums, 2nd Edition*. CAB International, Oxon.

Brown, J., Wright, J.L., Kohl, R.A., 1977. Onion-seed yield and quality as affected by irrigation management. *Agronomy Journal* 69, 369–372.

Caron, D.M., Lederhouse, R.C., Morse, R.A., 1975. Insect pollinators of onion in New York State. *Hortscience* 10, 273–274.

Carvalheiro, L.G., Seymour, C.L., Veldtman, R., Nicolson, S.W., 2010. Pollination services decline with distance from natural habitat even in biodiversity-rich areas. *Journal of Applied Ecology* 47, 810–820.

Cowling, R.M., 1986. *A description of the Karoo Biome Project*. South African National Scientific Programmes Report No. 122. Pretoria.

Cowling, R.M., Roux, P.W., Pieterse, A.I.H., 1986. *The karoo biome: a preliminary synthesis. Part 1 - physical environment*. South African National Scientific Programmes Report No 124, Pretoria.

Currah, L., Ockendon, D.J., 1983. Onion pollination by blowflies and honeybees in large cages. *Annals of Applied Biology* 103, 497–506.

Currah, L., Ockendon, D.J., 1984. Pollination activity by blowflies and honeybees on onions in breeders' cages. *Annals of Applied Biology* 105, 167–176.

Dean, W.R.J., Milton, S.J., 1995. Plant and invertebrate assemblages on old fields in the arid southern Karoo, South Africa. *African Journal of Ecology* 33, 1–13.

Diekötter, T., Kadoya, T., Peter, F., Wolters, V., Jauker, F., 2010. Oilseed rape crops distort plant–pollinator interactions. *Journal of Applied Ecology* 47, 209–214.

Eardley, C.D., 1996. The genus *Scrapter* Lepeletier & Serville (Hymenoptera: Colletidae). *African Entomology* 4, 37–92.

Free, J.B., 1993. Alliaceae, in: *Insect pollination of crops*. Academic Press, London, UK, pp. 115–121.

Garibaldi, L.A., Aizen, M.A., Klein, A.-M., Cunningham, S.A., Harder, L.D., 2011. Global growth and stability of agricultural yield decrease with pollinator dependence. *Proceedings of the National Academy of Sciences of the United States of America* 108, 5909–5914.

Gary, N.E., Witherell, P.C., Lorenzen, K., Marston, J.M., 1977. The interfield distribution of honey bees foraging on carrots, onions and safflower. *Environmental Entomology* 6, 637–640.

Gary, N.E., Witherell, P.C., Marston, J.M., 1972. Foraging range and distribution of honey bees used for carrot and onion pollination. *Environmental Entomology* 1, 71–78.

Gess, S.K., 1996. *The pollen wasps: ecology and natural history of the Masarinae*. Harvard University Press, Cambridge.

Gess, S.K., 2001. *The Karoo, its insect pollinators and the perils which they face*. International Pollinator Initiative (IPI) Case Study.

Greenleaf, S.S., Kremen, C., 2006. Wild bee species increase tomato production and respond differently to surrounding land use in North California. *Biological Conservation* 133, 81–87.

Hagler, J.R., 1990. Honey bee (*Apis mellifera* L.) response to simulated onion nectars containing variable sugar and potassium concentrations. *Apidologie* 21, 115–121.

Hagler, J.R., Cohen, A.C., Loper, G.M., 1990. Production and composition of onion nectar and honey bee (Hymenoptera: Apidae) foraging activity in Arizona. *Environmental Entomology* 19, 327–331.

Hawthorn, L.R., 1951. Studies of soil moisture and spacing for seed crops of carrots and onions. *United States Department of Agriculture, Circular 892*, 26p.

Hepburn, H.R., 1993. Swarming, absconding and migration in southern African bees. *South African Bee Journal* 65, 61–66.

Hepburn, H.R., Radloff, S.E., 1998. *Honeybees of Africa*. Springer Verlag, Berlin.

Hesse, A.J., 1938. A revision of the Bombyliidae (Diptera) of southern Africa. Part 1. *Annals of the South African Museum* 34, 1–1053.

Johannsmeier, M.F., 2001. *Beekeeping in South Africa, 3rd Edition*. Agricultural Research Council-Plant Protection Research Institute Handbook No 14.

Johannsmeier, M.F., 2005. Beeplants of the south-western Cape. *Plant Protection Research Institute Handbook No. 17*. Pretoria.

Klein, A.-M., Steffan-Dewenter, I., Tscharntke, T., 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society B: Biological Sciences* 270, 955–961.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274, 303–313.

Kremen, C., Williams, N.M., Bugg, R.L., Fray, J.P., Thorp, R.W., 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* 7, 1109–1119.

Kremen, C., Williams, N.M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences* 99, 16812–16816.

Kuhlmann, M., 2009. Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in southern Africa. *South African Journal of Botany* 75, 726–738.

le Roux, L., 2013. Reuse verliese nadat bye wegbly. Die Hoorn, 24 January, p.6.

Manning, J.C., Goldblatt, P., 1996. The *Prosoeca peringueyi* (Diptera: Nemestrinidae) pollination guild in southern Africa: long-tongued flies and their tubular flowers. *Annals of the Missouri Botanical Garden* 83, 67–86.

Mayer, D.F., Lunden, J.D., 2001. Honey bee management and wild bees for pollination of hybrid onion seed. *Acta Horticulturae* 561, 275–278.

McGregor, S.E., 1976. Common vegetables for seed and fruit, in: McGregor, S.E. (Ed.), *Insect pollination of cultivated crop plants*. United States Department of Agriculture, Washington.

Morandin, L.A., Winston, M.L., 2006. Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agriculture, Ecosystems and Environment* 116, 289–292.

Morandin, L.A., Winston, M.L., Abbott, V.A., Franklin, M.T., 2007. Can pastureland increase wild bee abundance in agriculturally intense areas? *Basic and Applied Ecology* 8, 117–124.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., De Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.

Olschewski, R., Tscharntke, T., Benítez, P.C., Schwarze, S., Klein, A.-M., 2006. Economic evaluation of pollination services comparing coffee landscapes in Ecuador and Indonesia. *Ecology And Society* 11, 7. (online http://www.ecologyandsociety.org/vol11/iss1/art7/)

Parker, F.D., 1982. Efficiency of bees in pollinating onion flowers. *Journal of the Kansas Entomological Society* 55, 171–176.

Picker, M.D., Midgley, J.J., 1996. Pollination by monkey beetles (Coleoptera: Scarabaeidae: Hopliini): flower and colour preferences. *African Entomology* 4, 7–14.

Rai, N., Rai, M., 2006. *Heterosis breeding in vegetable crops*. New India Publishing Agency, New Delhi.

Richards, A.J., 2001. Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Annals of Botany* 88, 165–172.

Ricketts, T.H., Daily, G.C., Ehrlich, P.R., Michener, C.D., 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences* 101, 12579–12582.

Silva, E.M., 1998. *The effect of onion (*Allium cepa *L.) flower characteristics on its pollination by the honey bee (*Apis mellifera *L.)*. MSc thesis, Washington State University.

Steffan-Dewenter, I., Tscharntke, T., 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121, 432–440.

Steyn, T., 2009. Bysiekte in tot 85% van korwe. Die Burger, 14 July, p.6.

Struck, M., 1994. Flowers and their insect visitors in the arid winter rainfall region of southern Africa: observations on permanent plots. Insect visitation behaviour. *Journal of Arid Environments* 28, 51–74.

Thompson, M., Vlok, J., Cowling, R.M., Cundill, S.L., Mundau, N., 2005. *A land transformation map for the Little Karoo*. GeoterraImage, Pretoria.

Tuell, J.K., Isaacs, R., 2009. Elevated pan traps to monitor bees in flowering crop canopies. *Entomologia Experimentalis et Applicata* 131, 93–98.

Vlok, J., Schutte-Vlok, A.L., 2010. *Plants of the Klein Karoo*. Umdaus Press, Pretoria.

Waller, G.D., 1974. Evaluating the foraging behavior of honey bees as pollinators of hybrid onions., in: *Proceedings of the Third International Pollination Symposium*. Prague, pp. 175–180.

Waller, G.D., Carpenter, E.W., Ziehl, O.A., 1972. Potassium in onion nectar and its probable effect on attractiveness of onion flowers to honey bees. *Journal of the American Society of Horticultural Science* 97, 535–539.

Waller, G.D., Gary, N.E., Chester Jr, S.T., Karim, M.S., Martin, J.H., Vaissière, B.E., 1985. Honeybee colony populations and foraging rates on onion seed-fields in Arizona. *Journal of Apicultural Research* 24, 93–101.

Westphal, C., Steffan-Dewenter, I., Tscharntke, T., 2003. Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters* 6, 961–965.

Whitehead, V.B., 1984. Distribution, biology and flower relationships of fideliid bees of southern Africa (Hymenoptera, Apoidea, Fideliidae). *South African Journal of Zoology* 19, 87–90.

Wilkaniec, Z., Giejdasz, K., Proszynski, G., Zdzislaw, W., Giejdasz, J., 2004. Effect of pollination on onion seeds under isolation by the mason bee (*Osmia rufa* L.) (Apoidea, Megachilidae) on the setting and quality of obtained seeds. *Journal of Apicultural Science* 48, 35–41.

Williams, I.H., Free, J.B., 1974. The pollination of onion (*Allium cepa* L.) to produce hybrid seed. *Journal of Applied Ecology* 11, 409–417.

Wills, A.B., North, C., 1978. Problems of hybrid seed production. *Acta Horticulturae* 83, 31–36.

Winfree, R., Williams, N.M., Dushoff, J., Kremen, C., 2007. Native bees provide insurance against ongoing honey bee losses. *Ecology Letters* 10, 1105–1113.

Woyke, H.W., 1981. Some aspects of the role of the honeybee in onion seed production in Poland. *Acta Horticulturae* 111, 91–98.

APPENDIX A – Details of the 18 commercial onion hybrid seed farms used for data collection

Table A.1: Site details of the 18 commercial onion hybrid seed farms used for data collection in 2009 and 2010.

Farm Code	Farm Name	Variety	Sampling Day	Sampling Year	Region	Area	Nearest Town	Field Size (ha)	Latitude (S)	Longitude (E)	Alt (m)
A	09VV	N	11-11	2009	KK	Middelplaas	De Rust	1.6	33°31'93.8''	22°29'66.3''	357
B	09VDR	N	08-11	2009	KK	De Rust	De Rust	2.1	33°29'61.8''	22°32'61.4''	433
C	09OvdW	K & L*	30-10	2009	KK	Rooirivier	De Rust	8.0	33°32'20.3''	22°49'61.9''	575
D	09JD	M	01-11	2009	KK	Middelplaas	De Rust	5.5	33°32'01.2''	22°32'06.1''	399
E	09JO	O	23-10	2009	sK	Prince Albert	Prince Albert	2.3	33°13'03.1''	21°51'66.2''	468
F	09JR	J	04-11	2009	KK	Buffelsklip	Uniondale	2.5	33°31'60.9''	22°53'99.2''	595
G	09FvdM	J	06-11	2009	KK	Buffelsklip	Uniondale	1.5	33°31'06.7''	22°54'14.4''	575
H	10VV	B	26-10	2010	KK	Middelplaas	De Rust	1.6	33°31'93.8''	22°29'66.3''	357
I	10JD	B & C*	18-10	2010	KK	Middelplaas	De Rust	5.5	33°32'01.2''	22°32'06.1''	399
J	10OvdW	D	01-11	2010	KK	Rooirivier	De Rust	1.7	33°31'96.0''	22°48'97.2''	570
K	10DCS	H	14-11	2010	sK	Klipbanksfontein	Sutherland	2.0	32°49'38.9''	20°27'89.3''	828
L	10BLR	E	07-11	2010	sK	Rondekop	Laingsburg	2.8	33°14'41.9''	20°55'00.6''	611
M	10DCF	F	13-11	2010	sK	Excelsoir	Laingsburg	6.2	33°08'20.6''	20°51'99.9''	701
N	10FdT	F	11-11	2010	sK	Buffelsrivier	Laingsburg	2.1	33°09'72.1''	20°52'60.7''	664
O	10FvdM	A	02-11	2010	KK	Buffelsklip	Uniondale	1.5	33°31'06.7''	22°54'14.4''	575
P	10BLL	E	06-11	2010	sK	Laingsburg	Laingsburg	3.7	33°13'57.9''	20°52'30.2''	635
Q	10JK	G	10-11	2010	sK	Volstruisfontein	Matjiesfontein	1.6	33°05'97.5''	20°28'64.2''	885
R	10BvA	I	08-11	2010	sK	Laingsburg	Laingsburg	3.3	33°12'53.8''	20°51'10.4''	642
^S	11LvdW	P	28/29-11	2011	KK	Oudemuragie	De Rust	-	-	-	-
^T	11PS	Q	30-11	2011	KK	De Rust	De Rust	-	-	-	-

KK = Klein Karoo & sK = southern Karoo, * Two male-sterile varieties were pollinated with one male-fertile variety in the same crop. ^Two extra farms used for nectar data collection not indicated on the map in Figure A.1

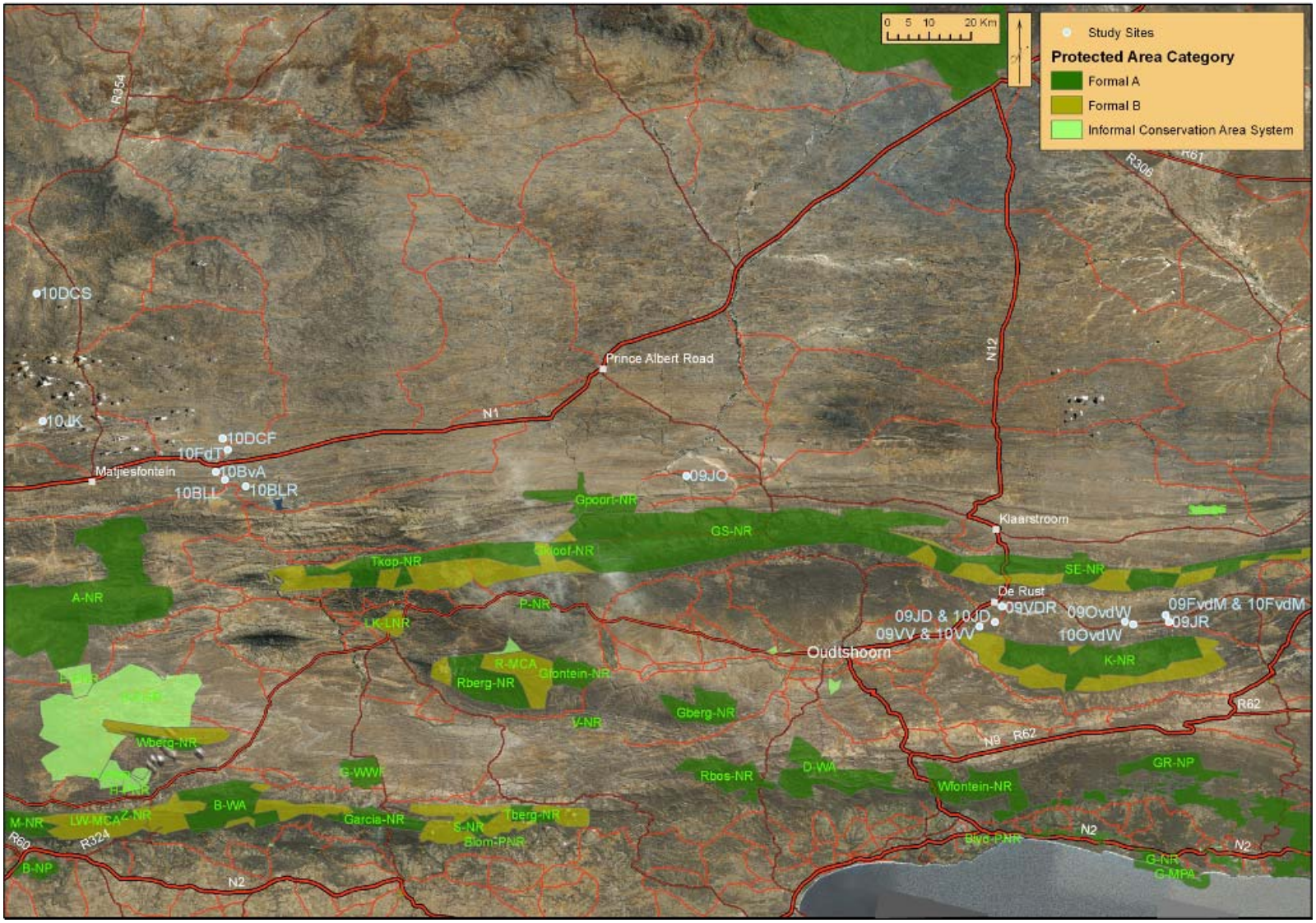


Figure A.1: The geographic locations of the 18 commercial onion hybrid seed crops used for data collection. All protected areas occupying an area greater than 1000 ha are also indicated (see Tables A.2 and A.3 for the category descriptions and the names of the protected areas).

The Protected Areas layer used in the map of Figure A.1 is a product of the National Protected Area Expansion Strategy 2008 (NPAES) which was a joint venture between SANParks and SANBI. The protected areas are categorized into three categories based on the level of protection they enjoy. Note that the formal protected area system dataset can be considered fairly reliable, but the Informal Conservation Area system data is known to contain significant errors and omissions.

Table A.2: Categorisation of protected areas

Type of Protected Area	Description
Formal A	
Forest Act Protected Area	Specially protected forest areas, forest nature reserves and forest wilderness areas declared in terms of the National Forests Act, 1998 (Act No. 84 of 1998)
Island Reserve	A sub-set of provincial nature reserves, which are islands administered by provinces in terms of provincial legislation
Marine Protected Area	An area declared as a marine protected area in terms of section 43 of the Marine Living Resources Act, 1998 (Act No. 18 of 1998)
National Park	An area declared in terms of the National Parks Act, 1976 (Act No. 57 of 1976), or in terms of Section 20 of the Protected Areas Amendment Act, 2004 (Act No. 31, 2004), including private areas declared under this legislation
Other national protected areas	A nature reserve other than a national park or special nature reserve, managed by a national organ of state or which falls under the jurisdiction of the Minister for any other reason
Provincial Nature Reserve	An area declared in terms of section 23 of Protected Areas Act, 2003 (No. 57 of 2003), or declared in terms of provincial legislation for conservation purposes, and which is managed by a provincial organ of state, including private areas declared under this legislation
Special nature reserve	An area which was a special nature reserve in terms of the Environment Conservation Act, 1989 (Act No. 73 of 1989), or an area declared in terms of section 18 of Protected Areas Act, 2003 (No. 57 of 2003)
World Heritage Site	A world heritage site declared in terms of the World Heritage Convention Act, 1999 (Act No. 49 of 1999)
MPA	Marine Protected Area, usually associated with an adjacent terrestrial protected area and managed by the same agency.
Formal B	
Mountain Catchment Area	An area declared in terms of the Mountain Catchment Areas Act, 1970 (Act No. 63 of 1970)
Local Nature Reserve	A nature reserve which is managed by a municipality, potentially of undefined legal status
National Botanical Garden	A reserve managed by the South African National Botanical Institute
Informal conservation area systems	
Unproclaimed Private Nature Reserves	Unproclaimed private Nature Reserves, Game Reserves or Game Farms

Table A.3: The protected areas that are indicated on the map of Figure A.1.

Abbreviation	Protected Area name	Protected area type	Category	Size of area (ha)
A-NR	Anysberg Nature Reserve	Provincial Nature Reserve	Formal A	67 597.9
B-NP	Bontebok National Park	National Park	Formal A	3 479.6
B-WA	Boosmansbos Wilderness Area	Provincial Nature Reserve	Formal A	14 655.6
D-WA	Doringrivier Wilderness Area	Provincial Nature Reserve	Formal A	9 524.0
E-PNR	Eyerpoort Private Nature Reserve	Private Nature Reserve	Informal Conservation Area system	3 612.0
Gberg-NR	Gamkaberg Nature Reserve	Provincial Nature Reserve	Formal A	9 591.6
Gpoort-NR	Gamkapoort Nature Reserve	Provincial Nature Reserve	Formal A	9 176.7
Gkloof-NR	Gamkaskloof Nature Reserve (Die Hel)	Provincial Nature Reserve	Formal A	4 446.8
Garcia-NR	Garcia Nature Reserve	Forest Act Protected Area	Formal A	6 461.2
GR-NP	Garden Route National Park	National Park	Formal A	39 764.6
G-MPA	Goukamma Marine Protected Area	Marine Protected Area	Formal A	3 396.5
G-NR	Goukamma Nature Reserve	Provincial Nature Reserve	Formal A	2 324.2
Gfontein-NR	Groenfontein Nature Reserve (Gamkaberg)	Provincial Nature Reserve	Formal A	5 218.2
GS-NR	Groot Swartberg Nature Reserve	Forest Act Protected Area	Formal A	79 743.2
G-MCA	Grootswartberg Mountain Catchment Area	Mountain Catchment Area	Formal B	8 699.7
G-WWF	Grootvadersbosch WWF land (Proposed reserve)	Provincial Nature Reserve	Formal A	4 617.8
H-PNR	Hasekraal Private Nature Reserve	Private Nature Reserve	Informal Conservation Area system	1 179.3
K-MCA	Kammanassie Mountain Catchment Area	Mountain Catchment Area	Formal B	22 132.2
K-NR	Kammanassie Nature Reserve	Forest Act Protected Area	Formal A	27 061.7
KS-MCA	Klein Swartberg Mountain Catchment Area	Mountain Catchment Area	Formal B	26 863.6
K-PNR	Klipfontein Private Nature Reserve	Private Nature Reserve	Informal Conservation Area system	2 540.5
LK-LNR	Ladismith-Kleinkaroo	Local Nature Reserve	Formal B	2 771.8
LE-MCA	Langeberg East Mountain Catchment Area	Mountain Catchment Area	Formal B	37 136.8
LW-MCA	Langeberg West Mountain Catchment Area	Mountain Catchment Area	Formal B	17 919.3
M-NR	Marloth Nature Reserve	Forest Act Protected Area	Formal A	11 235.9
P-NR	Paardenberg Nature Reserve	Forest Act Protected Area	Formal A	1 522.8
R-MCA	Rooiberg Mountain Catchment Area	Mountain Catchment Area	Formal B	12 545.1
Rberg-NR	Rooiberg Nature Reserve	Forest Act Protected Area	Formal A	12 842.4
Rbos-NR	Ruitersbos Nature Reserve	Forest Act Protected Area	Formal A	18 134.3
S-PGR	Sanbona Private Game Reserve	Private Nature Reserve	Informal Conservation Area system	49 574.7
S-NR	Spioenkop Nature Reserve	Provincial Nature Reserve	Formal A	1 256.8
S-PNR	Steenbokkie Private Nature Reserve	Private Nature Reserve	Informal Conservation Area system	3 493.4
SE-MCA	Swartberg East Mountain Catchment Area	Mountain Catchment Area	Formal B	3 348.1

Table A.3: continued

Abbreviation	Protected Area name	Protected area type	Category	Size of area (ha)
SS-GF	Sunnyside Game Farm	Private Nature Reserve	Informal Conservation Area system	~ 2 000.0
Tkop-NR	Towerkop Nature Reserve	Forest Act Protected Area	Formal A	18 984.9
Tberg-NR	Tygerberg Nature Reserve	Forest Act Protected Area	Formal A	2 800.5
V-NR	Vaalhoek Nature Reserve (Gamkaberg)	Provincial Nature Reserve	Formal A	1 336.9
Wberg-NR	Warmwaterberg Nature Reserve	Forest Act Protected Area	Formal A	2 693.5
Wfontein-NR	Witfontein Nature Reserve	Forest Act Protected Area	Formal A	14 349.7
Z-NR	Zuurberg Nature Reserve	Forest Act Protected Area	Formal A	1 232.2

APPENDIX B – Species checklist

Table B.1: Details of the taxonomists and collections where the various groups of insects were sent for identification and deposition.

Contact Person	Specimens	Deposited at
Dr. Connal Eardley Agricultural Research Council – Plant Protection Research Institute eardleyc@arc.agric.za	Apoidea	National Collection of Insects Agricultural Research Council – Plant Protection Research Institute Pretoria SOUTH AFRICA
Dr. Fredrich Gess Department of Entomology Albany Museum f.gess@ru.ac.za	Wasps	Albany Museum Rhodes University Grahamstown SOUTH AFRICA
Neal Evenhuis neale@bishopmuseum.org	Bombyliidae	*Small specimens (<5mm)* Bishop Museum 1525 Bernice Street Honolulu, Hawaii 96817 U.S.A.
Dr. Jonathan F. Colville j.colville@sanbi.org.za		*Large specimens (>5mm)* South African National Biodiversity Institute Kirstenbosch Research Centre Rhodes Drive Newlands Cape Town SOUTH AFRICA
Dr. Axel Ssymank ssymanka@t-online.de	Syrphidae	Bundesamt fr Naturschutz, II.2.2 Konstantinstrasse 110 53179 Bonn GERMANY
Dr. Marcia Couri courimarcia@gmail.com	Muscidae	Museu Nacional Quinta da Boa Vista, são Cristóvão Rio de Janeiro, RJ 20940-040 BRASIL
Pierfilippo Cerretti pierfilippocerretti@yahoo.it	Tachinidae	Centro Nazionale Biodiversità Forestale - Bosco Fontana Corpo Forestale dello Stato Via Carlo Ederle 16/a I-37100 Verona ITALY
Daniel Whitmore whitmore.daniel@gmail.com	Sarcophagidae	Natural History Museum of Denmark Universitetsparken 15 2100 Copenhagen, DENMARK

Table B.1: continued

Contact Person	Specimens	Deposited at
Prof. Knut Rognes knut.rognes@uis.no	Calliphoridae	University of Stavanger HUM / IFU / HL-huset NO-4036 Stavanger NORWAY
Dr. Jason Londt londtja@telkomsa.net	Asilidae	The Kwa-Zulu Natal Museum Private Bag 9070 Pietermaritzburg 3200 SOUTH AFRICA
Dr. Jonathan F. Colville j.colville@sanbi.org.za	Buprestidae	South African National Biodiversity Institute Kirstenbosch Research Centre Rhodes Drive, Newlands Cape Town SOUTH AFRICA
Prof. Marco A. Bologna bologna@bio.uniroma3.it	Meloidae	Dipartimento di Biologia Universita degli Studi "Roma Tre" Viale Marconi 446 00146 Roma ITALY
Dr. Jonathan F. Colville j.colville@sanbi.org.za	Scarabaeidae - Hoplinii	South African National Biodiversity Institute Kirstenbosch Research Centre Rhodes Drive, Newlands Cape Town SOUTH AFRICA
Mr. Riaan Stals stalsr@arc.agric.za Ms. Beth Grobbelaar grobbelaarb@arc.agric.za	Anthicidae Cantharidae Carabidae Coccinellidae Chrysomelidae Cerambycidae Elateridae Lycidae Nitidulidae Scarabaeidae - Cetoniinae - *Onthophagus* sp. Staphylinidae Tenebrionidae	National Collection of Insects Agricultural Research Council – Plant Protection Research Institute Pretoria SOUTH AFRICA
Dr. Pia Addison pia@sun.ac.za Stellenbosch University	Lepidoptera & Hemiptera	Department of Conservation Ecology and Entomology J.S. Marais Building Stellenbosch SOUTH AFRICA

Table B.2: The insect taxa identified from coloured pan traps (PT) and hand-collection (HC) efforts within onion hybrid seed crops in South Africa. Only those invertebrates spotted on onion umbels were hand-collected. The sexes are indicated where possible. Species marked with * are known alien species. (msp. indicates unidentified morpho-species)

Order	Family	Genus (species)	PT	HC
Hemiptera		*Spilostethus pandurus* Scopoli	✓	✓
		Hemiptera msp. 1	✓	✓
		Hemiptera msp. 2	✓	
		Hemiptera msp. 3	✓	
		Hemiptera msp. 4	✓	
		Hemiptera msp. 5	✓	
		Hemiptera msp. 6	✓	✓
		Hemiptera msp. 7	✓	
		Hemiptera msp. 8	✓	✓
		Hemiptera msp. 9	✓	
		Hemiptera msp. 10	✓	
		Hemiptera msp. 11	✓	✓
		Hemiptera msp. 12	✓	
		Hemiptera msp. 13	✓	
		Hemiptera msp. 14	✓	
		Hemiptera msp. 15	✓	
		Hemiptera msp. 16		✓
		Hemiptera msp. 17		✓
		Hemiptera msp. 18		✓
		Hemiptera msp. 19		✓
Coleoptera	Carabidae	*Cratognathus capensis* (Laporte, 1835)	✓	
		Harpalus sp. indet. A	✓	
		Harpalus sp. indet. B	✓	
		Harpalus sp. Indet. C	✓	
		Genus and species indeterminate	✓	
	Staphylinidae	Genus and species indeterminate		✓
	Scarabaeidae	*Heterochelus* sp. 1	✓	
		Heterochelus sp. 2	✓	✓
		Hopliini msp. 3	✓	
		Hopliini msp. 4	✓	
		Hopliini msp. 5	✓	
		Ischnochelus sp. 1	✓	✓
		Onthophagus sp. indet.	✓	
		Peritrichia sp. 1	✓	
		Polybaphes belteata balteata (DeGeer)		✓
		Scarabaeidae msp. 1		✓
	Buprestidae	Buprestidae msp. 1	✓	
		Buprestidae msp. 2	✓	
		Buprestidae msp. 3	✓	
		Buprestidae msp. 4	✓	
		Buprestidae msp. 5	✓	
		Buprestidae msp. 6	✓	
		Buprestidae msp. 7	✓	
		Buprestidae msp. 8	✓	

Table B.2: continued

Order	Family	Genus (species)	PT	HC
		Buprestidae msp. 9	✓	
		Buprestidae msp. 10	✓	
		Buprestidae msp. 11	✓	
		Buprestidae msp. 12	✓	
	Elateridae	Possibly *Aeolus* sp.	✓	
	Lycidae	*Lycus* cf. *ampliatus* Fahraeus, 1851		✓
	Cantharidae	*Afronycha* sp.		✓
	Nitidulidae	Genus and species indeterminate	✓	
	Coccinellidae	*Cheilomenes propinqua* (Mulsant, 1850)	✓	✓
		Exochomus cf. *flavipes* Thunberg, 1781	✓	✓
		**Harmonia axyridis* (Pallas, 1773)		✓
		**Hippodamia variegata* (Goeze, 1777)	✓	✓
		Lioadalia flavomaculata (DeGeer, 1778)	✓	✓
	Tenebrionidae	*Lagria (sensu lato)* sp. indet.		✓
	Meloidae	*Ceroctis aliena* (Péringuey)	✓	
		Ceroctis capensis (Linnaeus)	✓	
		Hycleus coecus (Thunberg)	✓	
		Hycleus decemguttatus (Thunberg) clx	✓	✓
		Hycleus tricolor (Thunberg)	✓	
		Prolytta pallidipennis (Haag-Rutenberg)	✓	
	Anthicidae	*Anthelephila cyanea* Hope		✓
	Cerambycidae	*Closteromerus claviger* (Dalman)		✓
	Chrysomelidae	*Altica cuprea* (Jacoby)	✓	
		Monolepta cruciata Guérin-Méneville		✓
		Monolepta melanogaster (Wiedemann)		✓
Diptera	Stratiomyiidae	*Hermetia illucens* (L.)	✓	
	Xylomyidae	*Xylomyi* sp.	✓	
	Tabanidae	Tabanidae sp. 1	✓	
	Asilidae	*Afroscleropogon dilutus* (Walker, 1851)	✓	✓
		Gonioscelis scapularis (Macquart)	✓	
		Rhacholaemus fisheri Londt	✓	
	Bombyliidae	*Bombomyia discoidea* (Fabricius, 1794)	✓	
		Corsomyza sp. 1	✓	
		Corsomyza sp. 2	✓	
		Exhyalanthrax sp.	✓	
		Hyperusia sp.	✓	✓
		Megapalpus capensis (Wiedemann)	✓	
		Pteraulax sp.	✓	
		Spogostylum incisurale (Macquart, 1840)	✓	
		Villa sp.	✓	
	Therevidae	Genus and species indeterminate	✓	
	Empididae	Empididae msp. 1	✓	
		Empididae msp. 2	✓	
	Syrphidae	*Eristalinus modestus* (Wiedemann, 1818)	✓	✓
		Eristalinus taeniops Wiedemann, 1818	✓	
		Eumerus obliquus (Fabricius, 1805)	✓	✓
		Eumerus sp. (probably undescribed)	✓	
		Eupeodes cf. corollae (Fabricius, 1794)	✓	
		Eupeodes corollae (Fabricius, 1794)	✓	✓

Table B.2: continued

Order	Family	Genus (species)	PT	HC
		Paragus sp. (subgenus Pandasyophthalmus)	✓	
		Simoides cf. crassipes (Fabricius, 1805)	✓	✓
		Syritta flaviventris Macquart, 1842	✓	✓
		Syritta vitripennis Bigot, 1885		✓
	Conopidae	*Thecophora* sp.	✓	
		Conopidae msp. 1	✓	
		Conopidae msp. 2	✓	
	Anthomyiidae	Anthomyiidae msp. 1	✓	
		Anthomyiidae msp. 2	✓	
		Anthomyiidae msp. 3	✓	
	Fanniidae	*Fannia* sp. 1	✓	
		Fannia sp. 2	✓	
		Fannia sp. 3	✓	
	Muscidae	*Atherigona* sp.	✓	
		Coenosia sp. 1	✓	
		Coenosia sp. 2	✓	
		Coenosia sp. 3	✓	
		Dimorphia tristis	✓	
		Gymnodia piliceps	✓	
		Gymnodia sp. 1	✓	
		Gymnodia sp. 2	✓	
		Gymnodia sp. 3	✓	
		Gymnodia sp. 4	✓	
		Hydrotaea sp.	✓	
		Limnophora obsignata	✓	
		Limnophora quaterna	✓	
		Limnophora sp. 1	✓	
		Limnophora sp. 2	✓	
		Limnophora sp. 3	✓	
		Lispe capensis	✓	
		Lispe leucospila	✓	
		Lispe sp. 1	✓	
		Lispe sp. 2	✓	
		Lispe sp. 3	✓	
		Musca sp. 1	✓	
		Musca sp. 2	✓	
		Musca spp.	✓	
		Muscidae msp. 1	✓	
		Muscidae msp. 2	✓	
		Muscidae msp. 3	✓	
		Muscidae msp. 4	✓	
		Muscidae msp. 5	✓	
		Muscidae msp. 6	✓	
		Muscidae msp. 17	✓	
		Muscidae msp. 28	✓	
		Muscidae msp. 34	✓	
		Muscina stabulans	✓	
		Neomyia peronii	✓	
	Calliphoridae	*Chrysomya albiceps* (Wiedemann)	✓	
		Chrysomya chlorophyga (Wiedemann)	✓	

Table B.2: continued

Order	Family	Genus (species)	PT	HC
		Chrysomya marginalis (Wiedemann)	✓	
		Lucilia cuprina (Wiedemann)	✓	
		Lucilia sericata (Meigen)	✓	
	Sarcophagidae	*Dolichotachina* sp.	✓	
		Wohlfahrtia pachytyli	✓	
		Sarcophagidae spp.	✓	
	Tachinidae	*Blepharella* sp.	✓	
		Chaetoria stylata	✓	
		Drino (*Palexorista*) sp.	✓	
		Leucostoma sp. (cf. *simplex*)	✓	
		Macquartia sp.	✓	
		Nemorilla sp. nov.	✓	
		Periscepsia sp.	✓	
		Phasia sp. nov.	✓	
		Phasia sp. 2	✓	
		Pretoriamyia sp.	✓	
		Thelyconychia sp. (cf. *solivaga*)	✓	
		Voria ruralis	✓	
Lepidoptera		Lepidoptera msp. 1	✓	✓
		Lepidoptera msp. 2	✓	
		Lepidoptera msp. 3	✓	
		Lepidoptera msp. 4	✓	
		Lepidoptera msp. 5	✓	
		Lepidoptera msp. 6	✓	
		Lepidoptera msp. 7	✓	
		Lepidoptera msp. 8	✓	
		Lepidoptera msp. 9	✓	
		Lepidoptera msp. 10	✓	
		Lepidoptera msp. 11	✓	
		Lepidoptera msp. 12	✓	
		Lepidoptera msp. 13	✓	
		Lepidoptera msp. 14	✓	
		Lepidoptera msp. 15	✓	
		Lepidoptera msp. 16	✓	
		Lepidoptera msp. 17		✓
		Lepidoptera msp. 18	✓	
		Lepidoptera msp. 19	✓	
		Lepidoptera msp. 20	✓	
		Lepidoptera msp. 21	✓	
		Lepidoptera msp. 22		
Hymenoptera	Ichneumonidae	Ichneumonidae sp. 1	✓	
		Ichneumonidae sp. 2	✓	
		Ichneumonidae sp. 3	✓	
		Ichneumonidae sp. 4	✓	
	Chalcididae	*Dirhinus* sp.	✓	
	Scoliidae	*Scolia chrysotricha* Burmeister	✓	
		Scoliidae msp.	✓	✓

Table B.2: continued

Order	Family	Genus (species)	PT	HC
	Tiphiidae	Tiphiidae msp.	✓	
	Pompilidae	Pompilidae msp.	✓	
	Vespidae	*Celonites promontorii* Brauns	✓	
		Delta caffer (L.)	✓	
		Polistes africanus Pal.de B.	✓	
		Polistes smithii Saussure	✓	
		Quartinia sp.	✓	
	Sphecidae	*Podalonia canescens* (Dahlbom)		✓
	Crabronidae	*Bembecinus ? argentifrons* (Smith)	✓	
		Bembecinus cinguliger (Smith)	✓	
		Cerceris latifrons Bingham	✓	
		Cerceris sp.		✓
		Dasyproctus bipunctatus Lep.&Br.	✓	
		Oxybelus		✓
		Palarus latifrons Kohl	✓	✓
		Philanthus triangulum (Fabricius)	✓	✓
		Pison transvaalensis Cameron	✓	
		Tachysphex	✓	
		Tachytes	✓	
		Trypoxylon	✓	
	Bradynobaenidae	*Apterogyna karroa* Péringuey	✓	✓
		Apterogyna kochi Invrea	✓	
	Colletidae	*Colletes* sp. 1	✓	
		Colletes sp. 2	✓	✓
		Colletes sp. 3	✓	
		Hylaeus sp.	✓	
		Hylaeus braunsi (Alfken)	✓	
		Hylaeus heraldicus (Smith)	✓	
	Halictidae	*Ceylalictus* sp.	✓	✓
		Halictus sp. 1	✓	
		Halictus sp. 2	✓	
		Halictus sp. 3	✓	
		Halictus sp. 4	✓	✓
		Halictus sp. 5	✓	
		Halictus sp. 6	✓	
		Halictus sp. 7		✓
		Lasioglossum sp. 1	✓	
		Lasioglossum sp. 2	✓	
		Lasioglossum sp. 3	✓	
		Lasioglossum sp. 4	✓	
		Lasioglossum sp. 5	✓	
		Lasioglossum sp. 6	✓	
		Lasioglossum sp. 7	✓	
		Lasioglossum sp. 8	✓	
		Lasioglossum sp. 9	✓	
		Lasioglossum sp. 10	✓	
		Lasioglossum sp. 11	✓	
		Lasioglossum sp. 12	✓	
		Lasioglossum sp. 13	✓	
		Lasioglossum sp. 14	✓	

Table B.2: continued

Order	Family	Genus (species)	PT	HC
		Lasioglossum sp. 15	✓	
		Lasioglossum sp. 16	✓	
		Lasioglossum sp. 17	✓	
		Lasioglossum sp. 18	✓	
		Lasioglossum sp. 19	✓	
		Lasioglossum sp. 20	✓	
		Lasioglossum sp. 21	✓	
		Lasioglossum sp. 22	✓	
		Lipotriches sp.	✓	
		Nomia sp. 1	✓	
		Nomia sp. 2	✓	
		Nomia sp. 3	✓	
		Nomia sp. 4	✓	✓
		Nomioides sp.		
		Patellapis sp. 1	✓	
		Patellapis sp. 2	✓	
		Patellapis sp. 3	✓	
		Patellapis sp. 4	✓	
		Patellapis sp. 5		✓
		Pseudapis sp. 1		✓
	Megachilidae	*Fidelia villosa* Brauns	✓	
		Lithurgus spiniferus Cameron	✓	
		Megachile frontalis Smith	✓	
		Megachile semierma Vachal	✓	
		Megachile venusta Smith	✓	
		Osmiini sp.	✓	
		Othinosmia sp.	✓	
		Pseudoanthidium sp.	✓	
	Apidae	*Allodapula monticola* (Cockerell)	✓	✓
		Amegiila kaimosica (Cockerell)	✓	
		Amegiila obscuriceps (Friese)	✓	
		Amegiila niveata (Friese)	✓	
		Anthophora indet.	✓	
		Anthophora labrosa Friese	✓	
		Anthophora praecox Friese	✓	
		Braunsapis ? vitrea (Vachal)	✓	
		Braunsapis sp.	✓	
		Tetraloniella braunsiana (Friese)	✓	
		Tetraloniella nanula (Cockerell)	✓	
		Thyreus vachali (Friese)		✓
		Xylocopa caffra (Linnaeus)		✓